



US009085605B2

(12) **United States Patent**
Liu et al.

(10) **Patent No.:** **US 9,085,605 B2**
(45) **Date of Patent:** **Jul. 21, 2015**

(54) **CHEMICAL SYNTHESIS AND ANTI-TUMOR AND ANTI-METASTATIC EFFECTS OF DUAL FUNCTIONAL CONJUGATE**

2005/0280268 A1 12/2005 Dehn et al.
2006/0236763 A1 10/2006 Terada et al.
2007/0120295 A1 5/2007 Zeyfang et al.
2007/0249707 A1 10/2007 Holton
2010/0099644 A1 4/2010 Lee et al.

(75) Inventors: **Gang Liu**, Xuanwu District Beijing (CN); **Nan Zhao**, Xuanwu District Beijing (CN); **Yao Ma**, Xuanwu District Beijing (CN)

FOREIGN PATENT DOCUMENTS

CN 1609118 A 4/2005
CN 1712399 A 12/2005
WO WO94/00156 A1 1/1994
WO WO97/10259 A1 3/1997
WO WO2008/080291 A1 7/2008
WO WO2008/107174 A1 9/2008
WO WO2009/111271 A1 9/2009
WO WO2010/091650 A1 8/2010

(73) Assignee: **Shenzhen Salubris Pharmaceuticals Co., Ltd.**, Guangdong Province (CN)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/699,869**

(22) PCT Filed: **May 27, 2011**

(86) PCT No.: **PCT/CN2011/074817**

§ 371 (c)(1),
(2), (4) Date: **Feb. 11, 2013**

(87) PCT Pub. No.: **WO2011/147330**

PCT Pub. Date: **Dec. 1, 2011**

(65) **Prior Publication Data**

US 2013/0143826 A1 Jun. 6, 2013

(30) **Foreign Application Priority Data**

May 27, 2010 (CN) 2010 1 0184541

(51) **Int. Cl.**

A61K 38/06 (2006.01)

A61K 38/07 (2006.01)

C07K 5/02 (2006.01)

A61K 31/337 (2006.01)

A61K 47/48 (2006.01)

C07D 405/12 (2006.01)

C07D 409/12 (2006.01)

C07D 305/14 (2006.01)

(52) **U.S. Cl.**

CPC **C07K 5/0215** (2013.01); **A61K 31/337** (2013.01); **A61K 47/48023** (2013.01); **A61K 47/48038** (2013.01); **A61K 47/48338** (2013.01); **C07D 305/14** (2013.01); **C07D 405/12** (2013.01); **C07D 409/12** (2013.01)

(58) **Field of Classification Search**

CPC **A61K 38/00**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,317,771 A 3/1982 Shiba et al.
4,606,857 A 8/1986 Hasegawa et al.
4,801,578 A 1/1989 Monsigny et al.
6,191,290 B1 2/2001 Safavy
7,459,281 B2 12/2008 Salamone et al.
2005/0143446 A1 6/2005 Holton
2005/0182098 A1 8/2005 Holton et al.

Gang Liu, Chemical and biological research of anti-tumor drug paclitaxel and immunoenhancer muramyl dipeptide conjugate. Chinese Doctoral Dissertation & Master's Thesis Full-text Database (Doctor) Medicine and Health Science, Nov. 15, 2006.*

Sporn et al., Chemoprevention of Cancer, Carcinogenesis, 2000, vol. 21, 525-530.*

Auerbach et al., Cancer and Metastasis Reviews, 2000, 19, pp. 167-172.*

Gura, Science, 1997, 278 (5340): 1041-1042, encloses 1-5.*

Jain, Scientific American, Jul. 1994, 58-65.*

Cancer Drug Design and Discovery Neidle, Stephen, ed. (Elsevier/Academic Press, 2008) p. 431.*

Liu et al., CN1712399, published on Dec. 28, 2005, English abstract translation.*

Paclitaxel, European Pharmacopoeia 7.0 Jan. 2009: 1794, p. 2657.
Docetaxel Trihydrate, European pharmacopoeia 7.0, Jan. 2010:2449, p. 1881-1882.

A. Galelli, P. Lefrancier, and L. Chedid; Colony-stimulating activity induced by synthetic muramyl peptides: variation with chemical structure and association with anti-infectious activity; Infection and Immunity; 1984, 46, 495-500.

A. H. Ding, F. Porteu, E. Sanchez, and C.F. Nathan; Shared actions of endotoxin and taxol on TNF receptors and TNF release; Science; 1990, 20, 370-372.

A. M. Kolodziejczyk, A. S. Kolodziejczyk, S. Stoev; New convenient synthesis of immunostimulating peptides containing meso-diaminopimelic acid Syntheses of FK-565 and FK-156; International Journal of Peptide and Protein Research; 1992, 39(4), 382-387.

(Continued)

Primary Examiner — Marcela M Cordero Garcia

Assistant Examiner — Kaipeen Yang

(74) *Attorney, Agent, or Firm* — Finnegan, Henderson, Farabow, Garrett & Dunner, LLP

(57) **ABSTRACT**

The present invention discloses chemical synthesis, anti-tumor and anti-metastatic effects of a dual functional conjugate as shown by formula I. Specifically, paclitaxel or docetaxol is linked with muramyl dipeptide derivative to form a conjugate, thus dual anti-tumor and anti-metastatic effects are achieved by combination of chemotherapy and immunotherapy. The present invention also discloses that paclitaxel or docetaxol and muramyl dipeptide derivative conjugate is synthesized by combination of solid-phase and solution-phase synthesis, and said conjugate can be used in manufacture of anti-tumor medicaments as proved by reliable bioassays.

35 Claims, 35 Drawing Sheets

(56)

References Cited

OTHER PUBLICATIONS

- Adam A., Lederer E.; Muramyl peptides: immunomodulators, sleep factors, and vitamins; *Med. Res. Rev.*, 1984, 4(2), 111-152.
- Adam A., Petit J. F., Chedid L.; Influence of a synthetic adjuvant (MDP) on qualitative and quantitative changes of serum globulins; *Immunology*; 1978, 35(6), 963-970.
- Adam A., Ciorbaru, R., Ellouz, F., Petit, J.F. and Lederer, E.; Adjuvant activity of monomeric bacterial cell wall peptidoglycans; *Biochem. Biophys. Res. Commun.*; 1974, 56(6), 561-567.
- Akihito T. et al.. Muramyl dipeptide-lys stimulates the function of human dendritic cells. *Journal of leukocyte Biology*, 70(5), 2001, 723-729.
- Alexandre A. Vetcher, Marek Napierala, Ravi R. Iyer, Paul D. Chastain, Jack D. Griffith, and Robert D.; Wells sticky DNA, a long GAA•GAA•TTC triplex that is formed intramolecularly, in the sequence of intron 1 of the frataxin gene; *J. Biol. Chem.*; 2002, 277, 39217-39227.
- Allen, J. N.; Moore, S. A.; Wewers, M. D. Taxol enhances but does not induce interleukin 1 beta and tumor necrosis factor alpha production. *J. Lab. Clin. Med.* 1993, 122, 374-381.
- Almand, B.; Clark, J. I.; Nikitina, E.; van Beynen, J.; English, N. R.; Knight, S. C.; Carbone, D. P.; Gabrilovi, D. I. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J. Immunol.* 2001, 166, 678-689.
- Anton V. Gorbachen, Nancy A. Dilulio, and Robert L.; Fairchild IL-12 augments CD81 T cell development for contact hypersensitivity responses and circumvents Anti-CD154 antibody-mediated inhibition; *The Journal of Immunology*, 2001, 167, 156-162.
- B. Bragg and A. Matus; Phosphorylation determines the binding of microtubule-associated protein 2 (MAP2) to microtubules in living cells; *J. Cell Biol.*; 1991, 114 (4), 735-743.
- Balkwill, F. TNF-R in promotion and progression of cancer. *Cancer Metastasis Rev.* 2006, 25, 709-716.
- Bhalla, K.; Ibrado, A. M.; Tourkina, E.; Tang, C.; Mahoney, M. E.; Huang, Y. Taxol induced internucleosomal DNA fragmentation associated with programmed cell. *Leukemia* 1993, 7, 563-568.
- Byrd-Leifer, C. A.; Block, E. F.; Takeda, K. Akira, S.; Ding, A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur. J. Immunol.* 2001, 31, 2448-2457.
- C. L. Contel, N. Temime, D. J. Charron, and M. A. Parant; Modulation of lipopolysaccharide-induced cytokine gene expression in mouse bone marrow-derived macrophages by muramyl dipeptide; *The Journal of Immunology*; 1993, 150, 4541-4549.
- Carboni, J., Singh, C., Tepper, M.; Cancer Institute Workshop on Taxol and Taxus, Alenandria, V. A.; NCI, 1992.
- Cassidy, P. B.; Moos, P. J.; Kelly, R. C.; Fitzpatrick, F. A. Cyclooxygenase-2 induction by paclitaxel, docetaxel and taxane analogs in human monocytes and murine macrophages: structure-activity relationships and their implications. *Clin. Cancer Res.* 2002, 8, 846-855.
- Chomel J. J., Simon-Lavoine N., Thouvenot D., Valette M., Choay J., Chedid L., Aymard M.; Prophylactic and therapeutic effects of murabutide in OF1 mice infected with influenza A and B viruses; *International Journal of Immunopharmacology*; 1985, 7(3), 346-347.
- Christian Bogdan and Aihao Ding; Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor alpha and interleukin-1 in macrophages; *Journal of Leukocyte Biology*; 1992, 52, 119-121.
- David L. Morse, Heather Gray, Claire M. Payne, and Robert J. Gillies; Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells; *Mol Cancer Ther.*; 2005, 4, 1495-1504.
- Deborah A. Eppstein, Noeline E. Byars, Anthony C. Allison; New adjuvants for vaccines containing purified protein antigens; *Advanced Drug Delivery Reviews* 1990, 4, 233-253.
- Deutsch, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Sulfness, M.; Zalkow, L. H. Synthesis of congeners and prodrugs. 3. Water-soluble prodrugs of Taxol with potent antitumor activity. *J. Med. Chem.* 1989, 32, 788-792.
- Diaz-Montero, C. M.; Salem, M. L.; Nishimura, M. I.; Garrett-Mayer, E.; Cole, D. J.; Montero, A. J. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol. Immunother.* 2009, 58, 49-59.
- Dietrich F. M., Hochkeppel H. K., Lukas B.; Enhancement of host resistance against virus infections by MTP-PE, a synthetic lipophilic muramyl peptide—increased survival in mice and guinea pigs after single drug administration prior to infection, and the effect of MTP-PE on interferon levels in sera and lungs; *Int. J. Immunopharmacol.*; 1986, 8, 931-932.
- E. C. A. Darcissac, V. Vidal, M. Guillaume, J. J. Thebault, G. M. Bahr; Clinical tolerance and profile of cytokine induction in healthy volunteers following the simultaneous administration of IFN- α and the synthetic immunomodulator murabutide; *Journal of Interferon and Cytokine Research*; 2001, 21(9), 655-661.
- Edith C. A. Darcissac, George M. Bahr, Philippe R. Pouillart, Gilles J. Riveau, Monique A. Parant; Selective potentiation of cytokine expression in human whole blood by mirabutide, a muramyl dipeptide analogue; *Cytokine*, 1996, 8, 658-666.
- Ellouz F., Adam A., Ciorbaru R., et al; Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives; *Biochem. Biophys. Res. Commun.*; 1974, 59, 1319-1325.
- F. Audibert, L. Chédid, P. Lefrancier, J. Choay; Distinctive adjuvanticity of synthetic analogs of mycobacterial water-soluble components; *Cellular Immunology*; 1976, 21, 243-249.
- Fitzpatrick, F. A.; Wheeler, R. The immunopharmacology of paclitaxel (Taxol), docetaxel (Taxotere), and related agents. *Int. Immunopharmacol.* 2003, 3, 1699-1714.
- Gang Liu, Chemical and biological research of antitumor drug paclitaxel and immunoenhancer muramyl dipeptide conjugate, Chinese Doctoral Dissertation & Master's Thesis Full-text Database (Doctor) Medicine and Health Science, Nov. 15, 2006, 2006, No. 11E079-85, ISSN: 1671-6779.
- George M. Bahr, Edith Darcissac, Dorian Bevec, Peter Dukor, Louis Chedid; Immunopharmacological activities and clinical development of muramyl peptides with particular emphasis on murabutide; *International Journal of Immunopharmacology*; 1995 17(2), 117-131.
- George M. Bahr, Philippe R. Pouillart, Louis A. Chedid; Enhancement in vivo of the anti-inflammatory and antitumor activities of type I interferon by association with the synthetic immunomodulator murabutide; *Journal of Interferon and Cytokine Research*; 1996, 16(4), 297-306.
- George M. Bahr, Edith Darcissac, Philippe R. Pouillart, Louis A. Chedid; Synergistic effects between recombinant interleukin-2 and the synthetic immunomodulator murabutide: selective enhancement of cytokine release and potentiation of antitumor activity; *Journal of Interferon and Cytokine Research*; 1996, 16(2), 169-178.
- Gilles J. Riveau, Beatrice G. Brunel-Riveau, Françoise M. Audibert, Louis A. Chedid; Influence of a muramyl dipeptide on human blood leukocyte functions and their membrane antigens; *Cellular Immunology*; 1991, 134, 147-156.
- Grown, J.; O' Leary, M. The taxanes: and update. *Lancet* 2000, 355, 1176-1178.
- Gupta, G. P.; Massague, J. Cancer metastasis: building a framework. *Cell* 2006, 127, 679-695.
- He, W.; Liu, Q.; Wang, L.; Chen, W.; Li, N.; Cao, X. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. *Mol. Immunol.* 2007, 44, 2850-2859.
- Hong-Zhen Yang, Song Xu, Xue-Yan Liao, Suo-De Zhang, Zheng-Lun Liang, Bai-He Liu, Jin-Ye Bai, Chao Jiang, Jian Ding, Gui-Fang Cheng, and Gang Liu; A novel immunostimulator, N2-[α -O-Benzyl-N-(acetylmuramyl)-l-alanyl-d-isoglutaminyl]N6-trans-(m-nitrocinanoyl)-l-lysine, and its adjuvancy on the hepatitis B surface antigen; *J. Med. Chem.*; 2005, 48(16), 5112-5122.
- Horwitz, S. B.; Lothstein, L.; Manfredi, J. J.; Mellado, W.; Parness, J.; Roy, S. N.; Schiff, P. B.; Sorbara, L.; Zeheb, R. Taxol: mechanisms of action and resistance. *Ann. N.Y. Acad. Sci.* 1986, 466, 733-744.

(56)

References Cited

OTHER PUBLICATIONS

- Huang, B.; Lei, Z.; Zhao, J.; Gong, W.; Liu, J.; Chen, Z.; Liu, Y.; Li, D.; Yuan, Y.; Zhang, G. M.; Feng, Z. H. CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. *Cancer Lett.* 2007, 252, 86-92.
- Ichiro Azuma, Tsukasa Seya; Development of immunoadjuvants for immunotherapy of cancer; *International Immunopharmacology*; 2001, 1(7), 1229-1392.
- Joyce, J. A.; Pollard, J.W. Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* 2009, 9, 239-252.
- Kawasaki, K.; Akashi, S.; Shimazu, R.; Yoshida, T.; Miyake, K.; Nishijima, M. Mouse Toll-like receptor 4. MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. *J. Biol. Chem.* 2000, 275, 2251-2254.
- Kenji Namba, Eiko Yamamura, Hironobu Nitani, Tsuyoshi Otani, Ichiro Azuma; Romurtide, a synthetic muramyl dipeptide derivative, promotes megakaryocytopoiesis through stimulation of cytokine production in nonhuman primates with myelosuppression; *Vaccine*, 1997, 15(4), 405-413.
- Kim, S.; Takahashi, H.; Lin, W. W.; Descargues, P.; Grivennikov, S.; Kim, Y.; Luo, J. L.; Karin, M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* 2009, 457, 102-106.
- L. A. Chedid, M. A. Parant, F. M. Audibert, G. J. Riveau, F. J. Parant, E. Lederer, J. P. Choay, and P. L. Lefrancier; Biological activity of a new synthetic muramyl peptide adjuvant devoid of pyrogenicity; *Infection and Immunity*; 1982, 35, 417-424.
- Lee, L. F.; Schuerer-Maly, C. C.; Lofquist, A. K.; van Haaften-Day, C.; Ting, J. P.; White, C. M.; Martin, B. K.; Haskill, J. S. Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. *Cancer Res.* 1996, 56, 1303-1308.
- Li, X.; Yu, J.; Xu, S.; Wang, N.; Yang, H.; Yan, Z.; Cheng, G.; Liu, G. Chemical conjugation of muramyl dipeptide and paclitaxel to explore the combination of immunotherapy and chemotherapy for cancer. *Glycoconjugate J.* 2008, 25, 415-425.
- Liebes L., Walsh C. M., Chachoua A., et al; Modulation of monocyte functions by muramyl triphosphatidylethanolamine in a phase II study in patients with metastatic melanoma; *J. Natl. Cancer. Inst.*; 1992, 84, 694-699.
- Mansukhlal C. Wani, Harold Lawrence Taylor, Monroe E. Wall, Philip Coggon, Andrew T. McPhail; Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*; *J. Am. Chem. Soc.*; 1971, 93(9), 2325-2327.
- Manthey, C. L.; Qureshi, N.; Stutz, P. L.; Vogel, S. N. Lipopolysaccharide antagonists block Taxol-induced signaling in murine macrophages. *J. Exp. Med.* 1993, 178, 695-702.
- Mantovani, A.; Garlanda, C.; Allavena, P. Molecular pathways and targets in cancer-related inflammation. *Ann. Med.* 2010, 42, 161-170.
- Mehlen, P.; Puisieux, A. Metastasis: a question of life or death. *Nat. Rev. Cancer* 2006, 6, 449-458.
- Meyers Paul A., Schwartz Cindy L., et al; A randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate; *J. Clin. Oncol.*; 2005, 23(9), 2004-2011.
- Meyers, P. A. Muramyl tripeptide (mifamurtide) for the treatment of osteosarcoma. *Expert Rev Anticancer Ther.* 2009, 9, 1035-1049.
- Moos, P. J.; Muskardin, D. T.; Fitzpatrick, F. A. Effect of Taxol and taxotere on gene expression in macrophages: induction of the prostaglandin synthase-2 isoenzyme. *J. Immunol.* 1999, 162, 467-473.
- Nardin A. Lefebvre M. L., Labroquere K., Faure O., Abastado J. P.; Liposomal muramyl tripeptide phosphatidylethanolamine: targeting and activating macrophages for adjuvant treatment of osteosarcoma; *Current Cancer Drug Targets*; 2006, 6, 123-133.
- P. Walder, E. Buchar, Z. Machková, T. Vrba, M. Flegel, I. Janků, K. Mas'ek; Pharmacokinetic profile of the immunomodulating compound adamantylamide dipeptide (AdDP), a muramyl dipeptide derivative in mice; *Immuno-pharmacology and Immunotoxicology*, 1991, 13(1 and 2), 101-119.
- Pablo D. Becker, Ricardo S. Corral, Carlos A. Guzmán, Saul Grinstein; Adamantylamide dipeptide as effective immunoadjuvant in rabbits and mice; *Vaccine*; 2001, 19(32), 4579-4903.
- Parant, M. A.; Audibert, F. M.; Chedid, L. A.; Level, M. R.; Lefrancier, P. L.; Choay, J. P.; Lederer, E. Immunostimulant activities of a lipophilic muramyl dipeptide derivative and of desmuramylpeptidolipidanalogs. *Infect. Immun.* 1980, 27, 826-831.
- Peter B. Schiff and Susan B. Horwitz; Taxol stabilizes microtubules in mouse fibroblast cells; *Proc. Natl. Acad. Sci. USA*; 1980, 77(3), 1561-1565.
- Philippe R. Poullart, Francoise M. Audibert, Louis A. Chedid, Pierre L. Lefrancier, George M. Bahr; Enhancement by muramyl peptides of the protective response of interferon- α/β against encephalomyocarditis virus infection; *International Journal of Immunopharmacology*; 1996, 18(3), 183-192.
- Shoemaker, R. H. The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer* 2006, 6, 813-823.
- Sinha, P.; Celestins, V. K.; Bunt, S. K.; Albelda, S. M.; Ostrand-Rosenberg, S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J. Immunol.* 2007, 179, 977-983.
- Steeg, P. S. Tumor metastasis: mechanistic insights and clinical challenges. *Nat. Med.* 2006, 12, 895-904.
- Subbaramaiah, K.; Hart, J. C.; Norton, L.; Dannenberg, A. J. Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 and p38 mitogen-activated protein kinase pathways. *J. Biol. Chem.* 2000, 275, 14838-14845.
- Szajnik, M.; Szczepanski, M. J.; Czystowska, M.; Elishaev, E.; Mandapathil, M.; Nowak-Markwitz, E.; Spaczynski, M.; Whiteside, T. L. TLR4 signaling induced by lipopolysaccharide or paclitaxel regulates tumor survival and chemoresistance in ovarian cancer. *Oncogene* 2009, 28, 4353-4363.
- Tosjiyuki Harada, Shigeaki Ogura, Koichi Yamazaki, Ichiro Kinoshita, Tomoolth, Hiroshi Isobe, Katsushige Yamashiro, Hitoshi Dosaka-Akita, Masaharu Nishimura; Predictive value of expression of P53, Bcl-2 and lung resistance-related protein for response to chemotherapy in non-small cell lung cancers; *Cancer Science*; 2005, 94(4), 394-399.
- Tsavaris, N.; Kosmas, C.; Vadiaka, M.; Kanelopoulos, P. Boulamatsis, D. Immune changes in patients with advanced breast cancer undergoing chemotherapy with taxanes. *Br. J. Cancer* 2002, 87, 21-27.
- Wang, A. C.; Su, Q. B.; Wu, F. X.; Zhang, X. L.; Liu, P. S. Role of TLR4 for paclitaxel chemotherapy in human epithelial ovarian cancer cells. *Eur. J. Clin. Invest.* 2009, 39, 157-164.
- Wang, J.; Kobayashi, M.; Han, M.; Choi, S.; Takano, M.; Hashino, S.; Tanaka, J.; Kondoh, T.; Kawamura, K.; Hosokawa, M. MyD88 is involved in the signalling pathway for Taxol induced apoptosis and TNF-R expression in human myelomonocytic cells. *Br. J. Haematol.* 2002, 118, 638-645.
- Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. Plant antitumor agents. VI. Isolation and structure of Taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* 1971, 93, 2325-2327.
- Watson, J. M.; Kingston, D. G.; Chordia, M. D.; Chaudhary, A. G.; Rinehart, C. A.; Haskill, J. S. Identification of the structural region of Taxol that may be responsible for cytokine gene induction and cytotoxicity in human ovarian cancer cells. *Cancer Chemother. Pharmacol.* 1998, 41, 391-397.
- Wolfert Margreet A. et al. The origin of the synergistic effect of muramyl dipeptide with endotoxin and peptidoglycan. *J. Bio. Chem.* 277(42), 2002, 39179-39186.
- Wu, Y.; Zhou, B. P. Inflammation: a driving force speeds cancer metastasis. *Cell Cycle* 2009, 8, 3267-3273.
- Wu, Y.; Zhou, B. P. TNF- α /NF- κ B/Snail pathway in cancer cell migration and invasion. *Br. J. Cancer* 2010, 102, 639-644.
- Y. Osada, T. Otani, M. Sato, t. Une, K. Matsumoto, and H. Ogawa; Polymorphonuclear leukocyte activation by a synthetic muramyl dipeptide analog; *Infection and Immunity*; 1982, 38, 848-854.

(56)

References Cited

OTHER PUBLICATIONS

- Yang, L.; DeBusk, L. M.; Fukada, K.; Fingleton, B.; Green-Jarvis, B.; Shyr, Y.; Matrisian, L. M.; Carbone, D. P.; Lin, P. C. Expansion of myeloid immune suppressor Gr^{1b}CD11b⁺ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 2004, 6, 409-421.
- Yang, L.; Huang, J.; Ren, X.; Gorska, A. E.; Chytil, A.; Aakre, M.; Carbone, D. P.; Matrisian, L. M.; Richamond, A.; Lin, P. C.; Moses, H. L. Abrogation of TGF- β signaling in mammary carcinomas recruits Gr^{1b}CD11b⁺ myeloid cells that promote metastasis. *Cancer Cell* 2008, 13, 23-25.
- Young, M. R.; Lathers, D. M. Myeloid progenitor cells mediate immune suppression in patients with head and neck cancers. *Int. J. Immunopharmacol.* 1999, 21, 241-252.
- Yu Q, Gao J. X., He X. S., et al; Docetaxel induces apoptosis and regulates expressions of bax and bcl-2 protein in human breast carcinoma MCF-7 Cells; *Cancer Res. Pre. Treatment*, 2006, 33(6), 388-390.
- Zaks-Zilberman, M.; Zaks, T. Z.; Vogel, S. N. Induction of proinflammatory and chemokine genes by lipopolysaccharide and paclitaxel (Taxol) in murine and human breast cancer cell lines. *Cytokine* 2001, 15, 156-165.
- Bhattacharya, D.; Thio, C. L. Review of Hepatitis B Therapeutics. *Clin. Infect. Dis.* 2010, 51, 1201.
- Marie-Louise, M.; Qiang, D.; Maryline, M. B. Therapeutic vaccines and immune-based therapies for the treatment of chronic hepatitis B: Perspectives and challenges. *J. Hepatol.* 2011, 54, 6, 1286.
- Singh, M.; O' Hagan, D. Advances in vaccine adjuvants. *Nat. Biotechnol.* 1999, 17, 1075.
- Hui, C. K.; Lau, G. K. K. Advances in immunomodulating therapy of HBV infection. *Int. J. Med. Sci.* 2005, 2, 24-170.
- Kotani, S.; Watanabe, Y.; Kinoshita, F.; Shimonoto, T.; Morisaki, I.; Shiba, T.; Kusumoto, S.; Tarumi, Y.; Ikenaka, K. Biken J. Immunoadjuvant activities of synthetic N-acetyl-muramyl-peptides or -amino acids. 1975, 18, 105.
- Carelli, C.; Audibert, F.; Chedid, L. Persistent enhancement of cell-mediated and antibody immune responses after administration of muramyl dipeptide derivatives with antigen in metabolizable oil. *Infect. Immun.* 1981, 33, 312.
- Riveau, G.; Masek, K.; Parant, M.; Chedid, L. Central pyrogenic activity of muramyl dipeptide. *J. Exp. Med.* 1980, 152, 869.
- Ghada, M.; Anthony, W. C.; Jean-Philippe, D.; Gillian, M. B. Synthesis and Immunostimulating Properties of Lipophilic Ester and Ether Muramyl Peptide Derivatives. *J. Med. Chem.* 1996, 39, 4483.
- Parant, M.; Parant, F.; Chedid, L.; Yap, A.; Petit, J. F.; Lederer, E. Fate of the synthetic immunoadjuvant, muramyl dipeptide (14C-labelled) in the mouse. *Int. J. Immunopharmacol.* 1979, 1, 35.
- Chen, Y. Z.; Liu, G.; Senju, S.; Wang, Q.; Irie, A.; Haruta, M.; Matsui, M.; Yasui, F.; Kohara, M.; Nishimura, Y. Improved electrical performance of NILC poly-Si TFTs manufactured using H₂SO₄ and HCl solution. *Int. J. Immunopathol. Pharmacol.* 2010, 23, 165.
- Pristovsek, P.; Kidric, J.; Hadzi, D. Proposal of a 3D Peptide Pharmacophore of Muramyl Dipeptide-Type Immunostimulants. 1. Conformational Search of Active and Inactive Analogues. *J. Chem. Inf. Comput. Sci.* 1997, 37, 971.
- Pristovsek, P.; Kidric, J.; Hadzi, D. Bioactive Conformations of Small Peptides: A Method for Selection of Candidates Based on Conformations of Active and Inactive Analogs and Its Application to Muramyl Dipeptide. *J. Chem. Inf. Comput. Sci.* 1995, 35, 633.
- Inohara, N.; Ogura, Y.; Fontalba, A.; Gutierrez, O.; Pons, F.; Crespo, J.; Fukase, K.; Inamura, S.; Kusumoto, S.; Hashimoto, M.; Foster, S. J.; Moran, A. P.; Fernandez-Luna, J. L.; Nunez, G. Host Recognition of Bacterial Muramyl Dipeptide Mediated through NOD2. *J. Biol. Chem.* 2003, 278, 5509.
- Girardin, S. E.; Boneca, I. G.; Viala, J.; Chamaillard, M.; Labigne, A.; Thomas, G.; Philpott, D. J.; Sansonetti, P. J. Nod2 Is a General Sensor of Peptidoglycan through Muramyl Dipeptide (MDP). *J. Biol. Chem.* 2003, 278, 8869.
- Kobiyashi, K. S.; Chamaillard, M.; Ogura, Y.; Henegariu, O.; Inohara, N.; Nunez, G.; Flavell, R. A. Nod2-Dependent Regulation of Innate and Adaptive Immunity in the Intestinal Tract. *Science* 2005, 307, 731.
- Uehori, J.; Fukase, K.; Akazawa, T.; Uematsu, S.; Akira, S.; Funami, K.; Shingai, M.; Matsumoto, M.; Azuma, I.; Toyohsima, K.; Kusumoto, S.; Seya, T. Dendritic cell maturation induced by muramyl dipeptide (MDP) derivatives: Monoacylated MDP confers TLR2/TLR4 activation. *J. Immunol.* 2005, 174, 7096.
- Fidler, I. J.; Kleinerman, E. S. Therapy of cancer metastasis by systemic activation of macrophages: from the bench to the clinic. *Res. Immunol.* 144, 284-298 (1993).
- Killion, J. J.; Fidler, I. J. Therapy of cancer metastasis by tumoricidal activation of tissue macrophages using liposome-encapsulated immunomodulators. *Pharmacol. Ther.* 78, 141-154 (1998).
- Georg, G. I.; Chen, T. T.; Ojima, I.; Wyas, D. M., (eds.): *Taxane Anticancer Agents: Basic Science and Current Status*. (Developed from Symposia sponsored by the Divisions of Chemical Health and Safety, Medicinal Chemistry, and Organic Chemistry at the 207th National Meeting of the American Chemical Society, San Diego, California, Mar. 13-17, 1994.) [In: ACS Symp. Ser., 1995; 583], pp. 353.
- Schiff, P. B.; Fant, J.; Horwitz, S. B.: Promotion of microtubule assembly in vitro by taxol. *Nature* 277, 665-667 (1979).
- Ding, A. H.; Porteu, F.; Sanchez, E.; Nathan, C. F.: Shared action of endotoxin and taxol on TNF receptors and TNF release. *Science* 248, 370-372 (1990).
- Kikelj, D.; Pecar, S.; Kotnik, V.; Stalc, A.; Wraber-Herzog, B.; Simci, S.; Ihan, A.; Klamfer, L.; Povsic, L.; Grahek, R.; Suhadolc, E.; Hocevar, M.; Hoenig, H.; Rogi-Kohlenprath, R.: N-{trans-2-[2'-(Acetylamino)cyclohexyl]oxy]acetyl}-L-alanyl-D-glutamic acid: anovel immunologically active carbocyclic muramyl dipeptide analog. *J. Med. Chem.* 41, 530-539 (1998).
- Inohara, N.; Ogura, Y.; Fontalba, A.; Gutierrez, O.; Pons, F.; Crespo, J.; Fukase, K.; Inamura, S.; Kusumoto, S.; Hashimoto, M.; Foster, S. J.; Moran, A. P.; Fernandez-Luna, J. L.; Nunez, G.: Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J. Biol. Chem.* 278, 5509-5512 (2003).
- Uehara, A.; Yang, S.; Fujimoto, Y.; Fukase, K.; Kusumoto, S.; Shibata, K.; Sugawara, S.; Takada, H.: Muramyl dipeptide and diamino pimelic acid-containing desmuramyl peptides in combination with chemically synthesized toll-like receptor agonist synergistically induced production of interleukin-8 in a NOD2- and NOD1-dependent manner, respectively, in human monocytic cells in culture. *Cell. Microbiol.* 7, 53-61 (2005).
- Uehara, A.; Sugawara, S.; Kurata, S.; Fujimoto, Y.; Fukase, K.; Kusumoto, S.; Satta, Y.; Sasano, T.; Sugawara, S.; Takada, H.: Chemically synthesized pathogen-associated molecular patterns increase the expression of peptidoglycan recognition proteins via toll-like receptors, NOD1 and NOD2 in human oral epithelial cells. *Cell. Microbiol.* 7, 675-686 (2005).
- Yang, S.; Tamai, R.; Akashi, S.; Takeuchi, O.; Akira, S.; Sugawara, S.; Takada, H.: Synergistic effect of muramyl dipeptide with lipopolysaccharide or lipoteichoic acid to induce inflammatory cytokines in human monocytic cells in culture. *Infect. Immun.* 69, 2045-2053 (2001).
- Traub, S.; Kubasch, N.; Morath, S.; Kresse, M.; Hartung, T.; Schmidt, R. R.; Hermann, C.: Structural requirements of synthetic muropeptides to synergize with lipopolysaccharide in cytokine induction. *J. Biol. Chem.* 279, 8694-8700 (2004).
- Liu, G.; Zhang, S. D.; Xia, S. Q.; Ding, Z. K.: Solid-phase synthesis of muramyl dipeptide (MDP) derivatives using a multipin method. *Bioorg. Med. Chem. Lett.* 10, 1361-1363 (2000).
- Zhang, S. D.; Liu, G.; Xia, S. Q.; Wu, P.; Zhang, L.: "Meshed-bag-gathered-bunch" method for solid-phase synthesis of small molecular diverse compounds. *J. Comb. Chem.* 4, 131-137 (2002).
- Nicolaou, K. C.; Dai, W. M.; Guy, R. K.: Chemistry and biology of taxol. *Agnew. Chem., Int. Ed. Engl.* 33, 15-44 (1994).
- Ojima, I.; Lin, S.; Slater, J. C.; Wang, T.; Pera, P.; Bernacki, R. J.; Ferlini, C.; Scambia, G.: Syntheses and biological activity of C-3'-difluoromethyl-taxoids. *Bioorg. Med. Chem.* 8, 1619-1628 (2000).

(56)

References Cited

OTHER PUBLICATIONS

Kingston, D.G.I.: Recent advances in the chemistry of taxol. *J.Nat. Prod.* 63, 726-734 (2000).

Ojima, I., Lin, S., Wang, T.: Recent advances in the medicinal chemistry of taxoids with novel beta-amino acid side chains. *Curr.Med. Chem.* 6, 927-954 (1999) http://www.google.cn/books?hl=zh-CN&lr=&id=dzJ6W8waltAC&oi=fnd&pg=PA927&dq=Recent+advances+in+the+medicinal+chemistry+of+taxoids+with+novel+amino+acid+side+chains&ots=19IN5AqQvW&sig=VIHA_rwxlQjCwQtDaSayBOzvU24&redir_esc=y.

Matsumoto, K., Ogawa, H., Kusama, T., Nagase, O., Sawaki, N., Inage, M., Kusumoto, S., Shiba, T., Azuma, I.: Stimulation of non-specific resistance to infection induced by 6-O-acyl muramyl dipeptide analogs in mice. *Infect. Immun.* 32, 748-785 (1981).

Azuma, I., Okumura, H., Saiki, I., Kiso, M., Hasegawa, A., Tanio, Y., Yamamura, Y.: Adjuvant activity of carbohydrate analogs of N-acetylmuramyl-L-alanyl-D-isoglutamine on the induction of delayed-type hypersensitivity to azobenzenearsonate-N-acetyl-L-tyrosine guinea pigs. *Infect. Immun.* 33, 834-839 (1981).

Yang, H.Z., Xu, S., Liao, X.Y., Zhang, S.D., Liang, Z.L., Liu, B.H., Bai, J.Y., Jiang, C., Ding, J., Cheng, G.F., Liu, G.: A novel immunostimulator, N2-[α -O-Benzyl-N-(acetylmuramyl)-L-

alanyl-D-isoglutaminyl]-N6-trans-(m-nitrocinnamoyl)-L-lysine, and its adjuvancy on the hepatitis B surface antigen. *J. Med. Chem.* 48, 5112-5122 (2005).

Bourzat, J.D., Commercon, A.: A practical access to chiral phenylisoserinates, preparation of Taxotere analogs. *Tetrahedron Lett.* 34, 6049-6052 (1993).

Lin, S., Fang, K., Hashimoto, M., Nakanishi, K., Ojima, I.: Design and synthesis of a novel photoaffinity taxoid as a potential probe for the study of paclitaxel-microtubules interactions. *Tetrahedron Lett.* 41, 4287-4290 (2000).

Gross, P.H., Rimpler, M.: Muramyl peptides. 1. Stereochemical-pure derivatives on muramic and isomuramic acids. *Liebigs Ann. Chem.* 1, 37-45 (1986).

Denis, J.N., Greene, A.E., Guenard, D., Gueritte-Voegelein, F., Mangatal, L., Potier, P.: Highly efficient, practical approach to natural taxol. *J. Am. Chem. Soc.* 110, 5917-5919 (1988).

English abstract for CN1712399A, 2005.

English abstract for CN1609118A, 2005.

English abstract: Gang Liu, Chemical and biological research of anti-tumor drug paclitaxel and immunoenhancer muramyl dipeptide conjugate, Chinese Doctoral Dissertation Master's Thesis Full-text Database (Doctor) Medicine and Health Science, Nov. 15, 2006, No. 11E079-85, ISSN: 1671-6779.

* cited by examiner

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro																	
NSC: D -- 747905/1			Experiment ID: 0808NS92						Test Type: 08				Units: molar				
Report Date : 5 October 2008			Test date: 18 August 2008						QNS :				MC :				
COMI : T-M-220 (75984)			Stain reagent: SRB Dual-Pass Related						SSPL				OX3W				
Concentration Log10																	
Panel / cell line	Time		Mean optical densities						Percent Growth						GI50	TGI	LC50
	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	-7.5	-6.5	5.5	-4.5					
Leukemia																	
CCRF-CEM	0.221	1.127	0.291	0.283	0.246	0.229	0.243	8	7	3	1	4	<1.04E-8	> 3.25E-5	>	3.25E-5	
HL-60(TB)	0.702	2.368	0.634	0.738	0.592	0.571	0.625	-10	2	-16	-19	-18	<8.93E-9	3.06E-8	>	3.25E-5	
K-562	0.148	1.177	0.236	0.311	0.188	0.155	0.164	9	15	4	2	3	<9.66E-9	> 3.25E-5	>	3.25E-5	
MOLT-4	0.328	1.109	0.601	0.732	0.510	0.421	0.380	35	52	23	12	10	<1.88E-8	> 3.25E-5	>	3.25E-5	
RPMI-8226	0.349	1.043	0.343	0.334	0.304	0.283	0.278	-2	-4	1-3	-19	-10	<6.60E-9	1.67E-6	>	3.25E-5	
SR	0.224	0.475	0.230	0.234	0.206	0.196	0.195	2	4	-8	-13	2	<3.253E-9	.	>	3.25E-5	
Non-small cell lung cancer																	
A549/ATCC	0.185	1.105	0.927	0.418	0.353	0.343	0.318	81	25	18	17	14	1.16E-8	> 3.25E-5	>	3.25E-5	
EKVX	0.578	1.721	1.617	1.126	0.979	1.039	1.051	91	48	35	40	41	2.91E-8	> 3.25E-5	>	3.25E-5	
HOF-62	0.861	1.564	1.509	1.367	1.110	1.072	1.094	92	72	35	30	33	1.30E-7	> 3.25E-5	>	3.25E-5	
NCI-H226	0.835	1.587	1.520	1.396	1.212	1.308	1.272	91	75	50	63	58	> 3.25E-5	> 3.25E-5	>	3.25E-5	
NCI-H23	0.711	1.834	1.763	1.100	1.041	1.101	1.000	94	35	29	35	26	1.79E-8	> 3.25E-5	>	3.25E-5	
NCI-H322M	0.603	1.495	1.467	0.847	0.830	0.787	0.808	97	27	25	21	23	1.54E-8	> 3.25E-5	>	3.25E-5	
NCI-H460	0.201	1.566	1.291	0.372	0.256	3.253	0.242	80	12	5	5	3	9.01 E-9	> 3.25E-5	>	3.25E-5	
NCI H522	0.423	1.460	1.234	0.549	0.511	0.498	0.461	78	12	8	7	4	8.67E 9	> 3.25E-5	>	3.25E-5	
Colon Cancer																	
COLO 205	0.302	0.923	0.836	0.249	0.184	0.190	0.155	86	-18	-39	-37	-49	7.22E-9	2.19E-8	>	3.25E-5	
HCC-2998	0.459	1.314	1.108	0.535	0.369	0.476	0.533	76	9	-20	2	9	7.91 E-9		>	3.25E-5	
HCT-116	0.214	1.609	1.146	0.308	0.266	0.268	0.247	67	7	4	4	2	6.19E-9	> 3.25E-5	>	3.25E-5	
HCT-15	0.283	1.346	1.290	1.171	0.858	0.466	0.448	95	84	54	17	15	4.10E-7	> 3.25E-5	>	3.25E-5	
Hi29	0.220	1.349	0.866	0.282	0.232	0.228	0.190	57	5	1	1	-14	4.49E-9	3.62E-6	>	3.25E-5	
KM12	0.208	0.966	0.767	0.258	0.202	0.226	0.181	72	6	-3	2	-13	7.00C-9		>	3.25E-5	
SW-520	0.182	1.212	0.986	0.537	0.531	0.604	0.565	78	34	34	41	37	1.43E-8	> 3.25E-5	>	3.25E-5	
CNS cancer																	
SF-268	0.380	1.123	0.986	0.639	0.550	0.538	0.516	82	35	23	21	18	1.54E-8	> 3.25E-5	>	3.25E-5	
SF-295	0.624	2.176	2.378	1.371	1.747	0.712	0.692	94	48	8	6	4	2.95E-8	> 3.25E-5	>	3.25E-5	
SF-530	0.774	1.923	1.772	0.881	0.400	3.400	0.440	87	9	-37	-37	-43	9.70E-9	5.17E-8	>	3.25E-5	
SNB-19	0.638	1.644	1.551	1.157	3.887	0.918	0.844	91	52	25	28	20	3.72E-8	> 3.25E-5	>	3.25E-5	
SNB-75	0.560	1.070	0.358	0.500	0.337	0.374	0.403	57	-u	-42	-36	-31	4.04E-9	2.07E-0	>	3.20E-5	
U251	0.213	0.988	0.816	0.377	0.328	0.316	0.325	78	20	15	13	14	9.92E-9	> 3.75E-5	>	3.25E-5	
Melanoma																	
LOX IMVI	0.254	1.326	0.944	0.540	0.522	0.600	0.628	64	27	25	32	35	7.81E-9	> 3.25E-5	>	3.25E-5	
MAUVE-3M	0.450	0.737	0.696	0.674	0.552	0.587	0.610	86	78	36	48	56		> 3.25E-5	>	3.25E-5	
M14	0.435	1.536	1.352	0.703	0.285	0.469	0.582	83	24	-35	3	13	1.19E-8		>	3.25E-5	
SK-MEL-2	0.569	1.431	1.334	0.660	0.573	0.642	0.654	89	11	,	8	10	1.02E-8	> 3.25E-5	>	3.25E-5	
SK-MEL-28	0.364	1.004	0.939	0.684	0.706	0.762	0.717	90	50	53	62	55		> 3.25E-5	>	3.25E-5	
SK-MEL-5	0.867	2.704	2.741	1.760	1.236	1.329	1.201	102	49	20	25	18	3.06E-8	> 3.25E-5	>	3.25E-5	
UACC-257	0.549	1.275	1.165	0.885	0.898	0.922	0.887	85	46	48	51	47		> 3.25E-5	>	3.25E-5	
UACC-62	0.590	1.736	1.471	0.921	0.779	0.872	0.967	77	29	16	25	33	1.18E-8	> 3.25E-5	>	3.25E-5	
Ovarian Cancer																	
IGROV1	0.128	0.427	0.334	0.198	0.164	0.177	0.150	69	23	12	16	7	8.43E-9	> 3.25E-5	>	3.25E-5	
OVCAR-3	0.303	0.823	0.755	0.311	0.226	0.225	0.190	87	1	-26	-26	-37	8.78E-9	3.67E-8	>	3.25E-5	
OVCAR-4	0.375	1.400	1.393	0.928	0.851	0.855	0.822	99	54	46	47	44	1.07E-7	> 3.25E-5	>	3.25E-5	
OVCAR-5	0.545	1.423	1.427	0.756	0.682	0.732	0.773	101	24	16	21	26	1.49E-8	> 3.25E-5	>	3.25F-5	
OVCAR-8	0.384	1.727	1.645	0.843	0.612	0.666	0.680	94	34	17	21	22	1.76E-8	> 3.25E-5	>	3.25E-5	
SK-OV-3	0.527	1.067	1.006	0.639	0.578	0.561	0.528	89	30	9	6		1.48E-8	> 3.25E-5	>	3.25E-5	
Renal cancer																	

Figure 1A

736-0	0.741	2.272	2.097	1.729	1.112	0.932	0.975	89	65	24	12	15	7.46E-8	> 3.25E-5	> 3.25E-5
A498	0.808	1.434	1.430	1.170	0.841	0.921	0.948	99	58	5	18	22	4.58E-8	> 3.25E-5	> 3.25E-5
ACHN	0.287	1.153	1.146	1.030	0.793	0.631	0.650	99	86	58	40	42	9.15E-7	> 3.25E-5	> 3.25E-5
CAKI-1	0.505	1.960	1.917	1.813	1.396	0.903	0.890	98	91	62	28	27	7.14E-8	> 3.25E-5	> 3.25E-5
RXF 393	0.432	0.793	0.746	0.516	0.345	3.377	0.384	87	23	-20	-13	-11	1.23E-8	1.11E-7	> 3.25E-5
SN12C	0.312	1.056	0.902	0.575	0.495	0.510	0.529	79	35	25	27	29	1.50E-8	> 3.25E-5	> 3.25E-5
TK-10	0.564	1.104	1.084	1.009	0.750	3.695	0.661	96	82	34	24	18	1.53E-7	> 3.25E-5	> 3.25E-5
UO-31	0.286	1.002	0.940	0.912	0.709	0.503	0.436	91	87	59	30	21	6.68E-7	> 3.25E-5	> 3.25E-5
Prostate Cancer															
DU-145	0.239	0.814	0.801	0.218	0.143	0.127	0.088	98	-9	-40	-47	-63	9.11E-9	2.68E-8	5.06E-6
Breast cancer															
MCF7	0.244	1.372	0.901	0.397	0.379	0.384	0.372	58	14	12	12	11	4.97E-9	> 3.25E-5	> 3.25E-5
NCI/ADR-RES	0.498	1.735	1.799	1.565	1.290	0.988	0.939	105	86	64	40	36	1.22E-6	> 3.25E-5	> 3.25E-5
MDA-MB-231	530	1.011	0.998	0.629	0.604	0.489	0.516	97	20	15	-8	-3	1.34E-8	1.50E-6	> 3.25E-5
/ATCCO.															
HS 576T	0.561	1.109	0.975	0.695	0.589	0.590	0.543	75	24	5	5	-3	1.03E-8	1.33E-5	> 3.25E-5
MDA-MR-435	0.483	2.065	1.419	0.272	0.290	0.434	0.504	59	-44	-40	-10	1	3.99E-9	> 3.25E-5	> 3.25E-5
BT-549	0.882	1.497	1.331	0.996	0.907	0.858	0.821	73	18	4	-3	-7	8.58E-9	1.28E-6	> 3.25E-5
T-47D	0.542	1.184	1.131	0.732	0.690	0.703	0.642	92	30	23	25	16	1.53E-8	> 3.25E-5	> 3.25E-5
MDA-MB-468	0.580	1.156	1.064	0.461	0.437	0.434	0.361	84	-21	-25	-25	-38	6.68E-9	2.07E-8	> 3.25E-5

Figure 1B

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro																		
NSC D – 747906/1			Experiment ID: 0808NS92						Test Type: 08					Units: molar				
Report Date : 5 October 2008			Test date: 18 aeryca 2008						QNS :					MC :				
COMI : T-M-222 (75985)			Stain reagent: SRB Dual-Pass Related						SSPL 0X3W									
Panel / cell line	Time		Concentration Log10						Percent Growth					GI50	TGI	LC50		
	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	7.5	-6.5	5.5	-4.5						
Leukemia																		
CCRF-CEM	0.221	1.127	0.291	0.283	0.246	0.229	0.243	8	7	3	1	2	3.25E-9	>	3.25E-5	>	3.25E-5	
HL-60(TB)	0.702	2.368	0.634	0.738	0.592	0.571	0.625	-10	2	-16	-19	-11	3.25E-9	>	3.25E-5	>	3.25E-5	
K-562	0.148	1.177	0.236	0.311	0.188	0.166	0.164	9	16	4	2	2	3.25E-9	>	3.25E-5	>	3.25E-5	
MOLT-4	0.328	1.109	0.601	0.732	0.510	0.421	0.380	35	52	23	12	7	3.25E-8	<	3.25E-9	>	3.25E-5	
RPMI-8226	0.349	1.043	0.343	0.334	0.304	0.283	0.278	-2	-4	-13	-19	-20	3.25E-9		6.96E-8	>	3.25E-5	
SR	0.224	0.457	0.230	0.234	0.206	0.196	0.195	2	4	-8	13	-13	3.25E-9			>	3.25E-5	
Non-small cell lung cancer																		
A549/ATCC	0.185	1.066	0.339	0.376	0.325	0.326	0.341	17	22	16	16	18	<3.25E-9	>	3.25E-5	>	3.25E-5	
EKVX	0.578	1.835	1.076	1.167	0.898	0.960	0.795	40	47	25	30	17	<3.25E-9	>	3.25E-5	>	3.25E-5	
HOF-62	0.861	1.601	1.249	1.365	1.098	1.057	1.054	52	68	32	26	26	1.30E-7	>	3.25E-5	>	3.25E-5	
NCI-H226	0.835	1.644	1.397	1.439	1.256	1.300	1.276	69	75	52	57	55	>3.25E-5	>	3.25E-5	>	3.25E-5	
NCI-H23	0.711	1.766	0.969	1.237	1.077	1.047	1.062	24	50	35	32	33	<3.25E-9	>	3.25E-5	>	3.25E-5	
NCI-H322M	0.603	1.446	0.810	0.863	0.750	0.731	0.650	24	31	17	15	6	<3.25E-9	>	3.25E-5	>	3.25E-5	
NCI-H460	0.201	1.660	0.282	0.364	0.267	0.261	0.278	6	11	4	4	5	<3.25E-9	>	3.25E-5	>	3.25E-5	
NCI H522	0.423	1.445	0.462	0.487	0.446	0.444	0.470	4	6	2	2	5	<3.25E-9	>	3.25E-5		3.25E-5	
Colon Cancer																		
COLO 205	0.302	0.862	0.200	0.246	0.171	0.142	0.139	-34	-19	-44	-53	-54	<3.25E-9	<	3.25E-9		1.57E-6	
HCC-2998	0.459	1.276	0.463	0.578	0.399	0.427	0.450		15	-13	-7	-2	<3.25E-9		1.09E-7	>	3.25E-5	
HCT-116	0.214	1.649	0.294	0.310	0.243	0.234	0.270	6	7	2	1	4	<3.25E-9	>	3.25E-6	>	3.25E-5	
HCT-15	0.283	1.377	1.259	1.269	0.865	0.477	0.411	89	90	53	18	12	<3.99E-7	>	3.25E-5	>	3.25E-5	
Hi29	0.220	1.287	0.227	0.260	0.216	0.213	0.211	1	4	-2	-3	-4	<3.25E-9		1.43E-7	>	3.25E-5	
KM12	0.208	1.006	0.229	0.216	0.199	0.216	0.201	3	1	-5	1	-3	<3.25E-9			>	3.25E-5	
SW-520	0.182	1.153	0.406	0.425	0.423	0.507	0.572	23	25	25	33	40	<3.25E-8	>	3.25E-5	>	3.25E-5	
CNS cancer																		
SF-268	0.380	1.122	0.540	0.556	0.463	0.486	0.393	22	24	11	14	2	<3.25E-9	>	3.25E-5	>	3.25E-5	
SF-295	0.624	2.106	0.787	1.492	0.611	0.588	0.537	11	59	-6	-6	-14			2.99E-5	>	3.25E-5	
SF-539	0.774	2.033	0.627	1.032	0.432	0.457	0.518	-19	20	-44	-41	-3	<3.25E-9		3.25E-8	>	3.25E-5	
SNB-19	0.638	1.593	0.911	1.135	0.816	0.842	0.858	29	52	19	21	23			3.25E-5	>	3.25E-5	
SNB-75	0.580	1.092	0.416	0.657	0.393	0.401	0.466	-28	15	-32	-31	-20	<3.25E-9			>	3.25E-5	
U251	0.213	1.032	0.368	0.377	0.302	0.321	0.326	19	20	11	13	14	<3.25E-9	>	3.25E-5	>	3.25E-5	
Melanoma																		
LOX IMVI	0.254	1.460	0.593	0.625	0.549	0.644	0.714	28	31	24	32	38	3.25E-9	>	3.25E-5	>	3.25E-5	
MAUVE-3M	0.450	0.712	0.510	0.544	0.509	0.563	0.610	23	36	22	43	61			3.25E-5	>	3.25E-5	
M14	0.435	1.507	0.464	0.724	0.318	0.414	0.630	3	27	-27	-5	18	<3.25E-9			>	3.25E-5	
SK-MEL-2	0.569	1.416	0.703	0.787	0.613	0.633	0.743	16	-27	5	7	21	<3.99E-9	>	3.25E-5	>	3.25E-5	
SK-MEL-28	0.364	0.988	0.624	0.645	0.685	0.683	0.653	42	5	51	51	46			3.25E-5	>	3.25E-5	
SK-MEL-5	0.867	2.659	1.109	1.544	0.923	0.926	1.042	13	3	3	3	10	<3.25E-9	>	3.25E-5	>	3.25E-5	
UACC-257	0.549	1.206	0.847	0.795	0.810	0.835	0.781	45	40	40	43	35	<3.25E-9	>	3.25E-5	>	3.25E-5	
UACC-62	0.590	1.855	0.926	0.970	0.855	0.900	0.965	27	21	21	24	30	<3.25E-9	>	3.25E-5	>	3.25E-5	
Ovarian Cancer																		
OVCAR-3	0.303	0.874	0.265	0.268	0.224	0.224	0.130	-13	-12	-26	-26	-57	<3.25E-9	<	3.25E-9		1.91E-5	
OVCAR-4	0.375	1.385	0.900	0.927	0.876	0.852	0.821	52	55	50	47	44	2.74E-7	>	3.25E-5	>	3.25E-5	
OVCAR-5	0.545	1.379	0.712	0.798	0.666	0.638	0.675	20	30	15	11	16	<3.25E-9	>	3.25E-5	>	3.25E-5	
OVCAR-8	0.384	1.628	0.711	0.807	0.588	0.590	0.695	26	34	16	17	25	<3.25E-9	>	3.25E-5	>	3.25E-5	
SK-OV-3	0.527	1.064	0.568	0.664	0.543	0.542	0.545	8	25	3	3	3	<3.25E-9	>	3.25E-5	>	3.25E-5	
Renal cancer																		
736-0	0.741	2.278	1.507	1.811	1.119	0.937	1.127	50	70	25	13	25			>	3.25E-5	>	3.25E-5

Figure 2A

A498	0.808	1.442	0.960	1.068	0.811	0.884	0.917	24	41	.	12	17	<3.25E-9	>	3.25E-5	>	3.25E-5
ACHN	0.287	1.156	1.040	1.073	0.801	0.650	0.626	87	90	59	42	39	1.09E-6	>	3.25E-5	>	3.25E-5
CAKI-1	0.505	2.028	1.683	1.822	1.319	0.857	0.577	77	86	53	23	5	4.21E-9	>	3.25E-5	>	3.25E-5
RXF 393	0.432	0.759	0.396	0.491	0.350	0.354	0.367	-8	18	-19	-19	-15	3.25E-9	.	>	3.25E-5	
SN12C	0.312	1.134	0.546	0.583	0.482	0.508	0.549	28	33	21	24	29	3.25E-9	>	3.75E-5	>	3.25E-5
TK-10	0.564	1.083	0.782	0.979	0.638	0.596	0.525	42	80	14	6	-7	3.25E-9	9.46E-6	>	3.25E-5	
UO-31	0.236	1.005	0.819	0.883	0.814	0.498	0.433	74	83	73	29	20	3.25E-9	>	3.25E-5	>	3.25E-5
Prostate Cancer																	
DU-145	0.239	0.864	0.175	0.192	0.129	0.139	0.112	-27	20	-46	-42	-53	<3.25E-9		3.25E-9		1.69E-5
Breast cancer																	
MCF7	0.244	1.279	1.347	0.352	0.335	0.353	0.363	10	10	9	11	11	<3.25E-9	>	3.25E-5	>	3.25E-5
NCI/ADR-RES	0.498	1.682	0.509	1.609	0.326	0.873	0.523	85	94	70	32	2	1.08E-6	>	3.25E-5	>	3.25E-5
MDA-MB-231	0.530	1.184	0.782	0.775	0.618	0.578	0.655	38	37	13	7	19	<3.25E-9	>	3.25E-5	>	3.25E-5
/ATCC																	
HS 576T	0.561	1.044	0.498	0.571	0.483	0.457	0.470	-11	2	-14	-19	-16	3.25E-9			>	3.25E-5
MDA-MR-435	0.483	1.883	0.095	0.099	0.128	0.215	0.343	-80	-80	-74	-56	-29	<3.25E-9	<	3.25E-9		
BT-549	0.882	1.349	0.831	0.986	0.773	0.746	0.680	-6	22	-12	-15	22	<3.25E-			>	3.25E-5
T-47D	0.542	1.151	0.664	0.701	0.630	0.665	0.728	20	26	14	-20	30	<3.25E-	>	3.25E-9	>	3.25E-5
MDA-MB-468	0.580	1.162	0.407	0.447	0.422	0.388	0.353	-30	-23	-27	-33		<	3.25E-9		3.25E-5	

Figure 2B

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro																	
NSC D - 747903/1			Experiment ID: 0808NS92						Test Type: 08					Units: molar			
Report Date : 5 October 2008			Test date: 18 August 2008						ONS :					MC :			
COMI : T-M-213 (75982)			Stain reagent: SRB Dual-Pass Related						SSPL					OX3W			
Concentration Log10																	
Time		Mean optical densities						Percent Growth									
Panel / cell line	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	7.5	-6.5	5.5	-4.5	GI50	TGI	LC50		
Leukemia																	
CCRF-CEM	0.221	1.118	1.069	0.277	0.253	0.260	0.332	95	6	4	4	12	1.04E-8	>	3.25E-5	>	3.25E-5
HL-60(TB)	0.702	2.185	2.065	0.748	0.624	0.663	0.669	92	3	-11	-6	-5	9.63E-9	>	5.37E-8	>	3.25E-5
K-562	0.148	1.151	0.977	0.296	0.225	0.223	0.207	83	15	8	7	6	9.82E-9	>	3.25E-5	>	3.25E-5
MOLT-4	0.328	1.198	1.206	0.645	0.476	0.426	0.412	101	36	17	11	10	2.00E-8	>	3.25E-5	>	3.25E-5
RPMI-8226	0.349	1.015	0.730	0.340	0.332	0.320	0.331	57	-3	-5	-8	-5	4.28E-9	>	2.94E-8	>	3.25E-5
SR	0.224	0.544	0.460	0.231	0.221	0.227	0.218	74	2	-2	1	-3	6.97E-9	.	>	3.25E-5	
Non-small cell lung cancer																	
A549/ATCC	0.185	0.923	0.818	0.357	0.280	0.280	0.316	86	23	13	13	18	1.21E-8	>	3.25E-5	>	3.25E-5
EKVX	0.578	1.627	1.567	1.085	0.946	0.959	1.001	94	48	35	36	40	2.99E-8	>	3.25E-5	>	3.25E-5
HOF-62	0.861	1.576	1.484	1.309	1.046	0.992	1.049	87	63	26	18	26	7.15E-8	.	325E-5	.	3.25E-5
NCI-H226	0.835	1.667	1.619	1.449	1.294	1.442	1.458	94	74	bb	73	75	> 3.25E-5	>	325E-5	>	3.25E-5
NCI-H23	0.711	1.641	1.554	0.965	1.006	0.978	1.173	91	27	32	29	50	1.43E-8	>	3.25E-5	>	3.25E-5
NCI-H322M	0.603	1.504	1.488	0.922	0.881	0.849	1.001	98	35	31	27	44	1.90E-8	>	3.25E-5	>	3.25E-5
NCI-H460	0.201	1.542	1.098	0.343	0.253	0.252	0.255	67	11	4	4	4	6.48E-9	>	3.25E-5	>	3.25E-5
NCI H522	0.423	1.323	1.107	0.388	0.360	0.394	0.396	76	-8	-15	-7	-7	6.61E-9	.	2.59E-8	.	3.25E-5
Colon Cancer																	
COLO 205	0.302	0.744	0.647	0.194	0.211	0.216	0.178	78	-36	-30	-29	-41	5.73E-9	.	1.58E-8	.	3.25E-5
HCC-2998	0.459	1.196	1.083	0.498	0.354	0.487	0.573	85	5	-23	4	15	8.88E-9	.	.	.	3.25E-5
HCT-116	0.214	1.520	1.032	0.292	0.227	0.238	0.247	63	6	1	2	3	5.42E-9	>	325E-5	>	3.25E-5
HCT-15	0.283	1.256	1.230	1.176	0.768	0.445	0.366	97	92	50	17	9	3.22E-7	>	325E-5	>	3.25E-5
Hi29	0.220	1.281	0.740	0.243	0.208	0.197	0.232	49	3	-6	-10	1	< 3.25E-9	.	.	.	3.25E-5
KM12	0.208	0.956	0.714	0.264	0.229	0.252	0.280	68	7	3	6	10	6.39E-9	>	3.25E-5	>	3.25E-5
SW-520	0.182	1.059	0.344	0.456	0.450	0.529	0.566	75	31	31	40	44	1.22E-3	>	3.25E-5	>	3.25E-5
CNS cancer																	
SF-268	0.380	1.082	0.936	0.588	0.500	0.526	0.565	79	30	17	21	26	1.26E-8	>	3.25E-5	>	3.25E-5
SF-295	0.624	2.016	1.905	1.244	0.673	0.670	0.709	92	45	4	3	6	2.49E-8	>	3.25E-5	>	3.25E-5
SF-539	0.774	1.882	1.719	0.903	0.421	0.485	0.515	85d	12	-46	-40	-34	9.80E-9	.	519E-8	.	3.25E-5
SNB-19	0.638	1.499	1.397	1.030	0.747	0.801	0.813	88	45	13	19	20	2.54E-8	>	3.25E-5	>	3.25E-5
SNB-75	0.580	1.025	0.805	0.475	0.335	0.380	0.437	51	-18	-42	-35	-25	3.32E-9	.	1.77E-8	.	3.25E-5
U251	0/213	0/947	0.795	0.353	0.284	0.296	0.317	79	19	10	11	14	9.96E-9	>	3.25E-5	>	3.25E-5
Melanoma																	
LOX IMVI	0.254	1.351	0.987	0.584	0.526	0.596	0.668	67	30	25	31	38	9.32E-9	>	3.25E-5	>	3.25E-5
MAUVE-3M	0.450	0.730	0.666	0.521	0.515	0.556	0.573	77	25	23	38	44	1.08E-8	>	3.25E-5	>	3.25E-5
M14	0.435	1.468	1.278	0.773	0.397	0.843	0.642	82	33	-9	39	20	1.44E-8	.	.	.	3.25E-5
SK-MEL-2	0.569	1.343	1.268	0.585	0.528	0.561	0.659	90	2	-7	-1	12	9.31E-9	.	.	.	3.25E-5
SK-MEL-28	0.364	0.934	0.867	0.673	0.665	0.741	0.755	88	54	53	66	69	> 3.25E-5	>	3.25E-5	>	3.25E-5
SK-MEL-5	0.867	2.575	2.611	1.342	0.967	1.046	1.132	102	28	6	10	15	1.63E-8	>	3.25E-5	>	3.25E-5
UACC-257	0.549	1.115	1.054	0.767	0.770	0.808	0.769	89	39	39	46	39	1.93E-3	>	3.25E-5	>	3.25E-5
UACC-62	0.590	1.858	1.580	1.076	0.911	0.987	1.209	78	38	25	31	49	1.65E-8	>	3.25E-5	>	3.25E-5
Ovarian Cancer																	
OVCAR-3	0.303	0.803	0.692	0.296	0.247	0.265	0.282	78	-2	-19	-13	-7	7.21 E-9	.	3.03E-8	.	3.25E-5
OVCAR-4	0.375	1.357	1.332	0.949	0.911	0.959	0.871	97	58	55	59	51	> 3.25E-5	>	3.25E-5	>	3.25E-5
OVCAR-5	0.545	1.382	1.361	0.690	0.630	0.687	0.829	97	17	10	17	34	1.27E-8	>	3.25E-5	>	3.25E-5
OVCAR-8	0.384	1.492	1.436	0.758	0.566	0.571	0.678	95	34	16	17f	27	1.76E-8	>	3.25E-5	>	3.25E-5
SK-OV-3	0.527	1.007	0.941	0.577	0.492	0.495	0.556	86	10	-7	-6	6	9.76E-9	.	.	.	3.25E-5

Figure 3A

Renal cancer																	
736-0	0.741	2.178	2.070	1.770	1.047	0.921	1.211	93	72	21	12	33	8.73E-8	>	3.25E-5	>	3.25E-5
A498	0.808	1.352	1.334	1.157	0.723	0.883	0.987	97	64	-11	14	33	5.03E-8	.	>	3.25E-5	.
ACHN	0.287	1.114	1.139	1.061	0.758	0.603	0.586	103	94	57	38	36	7.62E-7	>	3.25E-5	>	3.25E-5
CAKI-1	0.505	1.870	1.822	1.750	1.397	0.900	0.813	97	91	63	29	23	8.58E-7	>	3.25E-5	>	3.25E-5
RXF 393	0.432	0.700	0.644	0.442	0.292	0.321	0.357	79	4	-33	-26	-17	7.87E-9	.	4.08E-8	>	3.25E-5
SN12C	0.312	1.073	0.958	0.581	0.509	0.536	0.582	85	35	26	29	35	1.64E-8	>	3.25E-5	>	3.25E-5
TK-10	0.564	1.092	1.059	0.939	0.816	0.681	0.662	94	71	48	22	19	2.57E-7	>	3.25E-5	>	3.25E-5
UO-31	0.286	0.957	0.892	0.895	0.710	0.508	0.443	90	91	63	33	23	8.88E-7	>	3.25E-5	>	3.25E-5
Prostate Cancer																	
DU-145	0.239	0.817	0.764	0.212	0.158	0.163	0.151	91	-11	-34	-32	-37	8.16E-9	.	2.52E-8	>	3.25E-5
Breast cancer																	
MCF7	0.244	1.177	0.701	0.348	0.315	0.281	0.210	49	11	8	4	-14	< 3.25E-9	.	5.38E-6	>	3.25E-5
NCI/ADR-RES	0.498	1.599	1.701	1.428	1.227	1.015	0.886	109	84	66	47	35	2.25E-6	>	3.25E-5	>	3.25E-5
MDA-MB-231	0.530	1.123	1.095	0.734	0.590	0.534	0.638	95	34	10	1	18	1.80E-8	>	3.25E-5	>	3.25E-5
/ATCC																	
HS 576T	0.561	0.978	0.920	0.655	0.564	0.583	0.553	86	22	1	5	-2	1.20E-8	.	1.93E-5	>	3.25E-5
MDA-MR-435	0.483	1.897	1.060	0.222	0.241	0.358	0.439	41	-54	-50	-26	-9	< 3.25E-9	.	8.75E-9	.	.
BT-549	0.882	1.497	1.318	1.075	0.916	1.011	0.924	71	31	5	21	7	1.09E-8	>	3.25E-5	>	3.25E-5
T-47D	0.542	1.137	1.056	0.672	0.712	0.702	0.658	36	22	29	27	19	1.19E-8	>	3.25E-5	>	3.25E-5
MDA-MB-468	0.580	1.187	1.089	0.491	0.454	0.445	0.417	84	-15	-22	-28	-28	7.13E-9	.	2.27E-8	>	3.25E-5

Figure 3B

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro																	
NSC D – 747904/1			Experiment ID: 0808NS392					Test Type: 08					Units: molar				
Report Date : 5 October 2008			Test date: 18 August 2008					ONS :					MC :				
COMI : T-M-219 (75983)			Stain reagent: SRB Dual-Pass Related					SSPL 0X3W									
Concentration Log10																	
Time		Mean optical densities						Percent Growth									
Panel / cell line	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	7.5	-6.5	5.5	-4.5	GI50	TGI	LC50		
Leukemia																	
CCRF-CEM	0.221	1.118	1.018	0.258	0.239	0.223	0.254	89	4	2	.	4	9.34E-9	>	3.25E-5	>	3.25E-5
HL-60(TB)	0.702	2.185	2.108	0.644	0.556	0.543	0.631	95	-8	-21	-23	-10	8.84E-9	>	2.70E-8	>	3.25E-5
K-562	0.148	1.151	0.877	0.285	0.193	0.182	0.188	73	14	4	3	4	7.87E-9	>	3.25E-5	>	3.25E-5
MOLT-4	0.328	1.198	1.040	0.602	0.446	0.423	0.425	82	31	14	-1	11	1.39E-8	>	3.25E-5	>	3.25E-5
RPMI-8226	0.349	1.015	0.581	0.327	0.298	0.281	0.277	35	-6	-15	-19	-21	< 3.25E-9	>	2.28E-8	>	3.25E-5
SR	0.224	0.544	0.393	0.205	0.177	0.194	0.202	53	-8	-21	-3	-10	3.61 E-9	>	2.36E-8	>	3.25E-5
Non-small cell lung cancer																	
A549/ATCC	0.185	0.923	0.679	0.332	0.280	0.288	0.314	67	20	13	14	17	7.46E-9	>	3.25E-5	>	3.25E-5
EKVX	0.578	1.627	1.518	1.037	0.928	0.950	0.992	90	44	33	35	39	2.37E-8	>	3.25E-5	>	3.25E-5
HOF-62	0.861	1.576	1.503	1.293	1.023	1.023	1.087	90	60	23	23	32	6.12E-8	>	3.25E-5	>	3.25E-5
NCI-H226	0.835	1.667	1.655	1.409	1.294	1.289	1.314	99	69	55	55	58	> 3.25E-5	>	3.25E-5	>	3.25E-5
NCI-H23	0.711	1.641	1.546	1.032	1.064	1.030	1.007	90	34	38	34	32	1.70E-8	>	3.25E-5	>	3.25E-5
NCI-H322M	0.603	1.504	1.474	0.881	0.816	0.800	0.758	97	31	24	22	17	1.66E-8	>	3.25E-5	>	3.25E-5
NCI-H460	0.201	1.542	0.922	0.321	0.271	0.251	0.288	54	9	5	4	6	3.94E-9	>	3.25E-5	>	3.25E-5
NCI H522	0.423	1.323	0.965	0.397	0.372	0.354	0.390	60	-6	-12	-16	-8	4.63E-9	>	2.63E-8	>	3.25E-5
Colon Cancer																	
COLO 205	0.302	0.744	0.612	0.195	0.129	0.135	0.135	70	-36	-57	-55	-55	5.04E-9	>	1.50E-8	>	1.50E-7
HCC-2998	0.459	1.196	0.983	0.620	0.468	0.477	0.424	71	22	1	2	-8	8.70E-9	>	5.68E-6	>	3.25E-5
HCT-116	0.214	1.520	0.945	0.296	0.229	0.226	0.333	56	6	1	1	9	4.29E-9	>	3.25E-5	>	3.25E-5
HCT-15	0.283	1.256	1.294	1.182	0.752	0.495	0.371	104	92	48	22	9	2.95E-7	>	3.25E-5	>	3.25E-5
Hi29	0.220	1.281	0.637	0.232	0.207	0.201	0.204	39	1	-6	-9	-8	< 3.25E-9	>	4.59E-8	>	3.25E-5
KM12	0.208	0.956	0.640	0.198	0.225	0.196	0.238	58	-5	2	-6	4	4.32E-9	.	.	>	3.25E-5
SW-520	0.182	1.059	0.682	0.369	0.392	0.415	0.508	57	21	24	27	37	5.12E-9	>	3.25E-5	>	3.25E-5
CNS cancer																	
SF-268	0.380	1.082	0.903	0.540	0.493	0.483	0.535	75	23	16	15	22	9.67E-9	>	3.25E-5	>	3.25E-5
SF-295	0.624	2.016	1.769	1.075	0.641	0.576	0.644	82	32	1	-8	1	1.44E-8	.	.	>	3.25E-5
SF-539	0.774	1.882	1.624	0.665	0.395	0.410	0.514	77	-14	-49	-47	-34	6.40E-9	>	2.27E-8	>	3.25E-5
SNB-19	0.638	1.499	1.349	0.913	0.758	0.752	0.830	83	32	14	13	22	1.43E-8	>	3.25E-5	>	3.25E-5
SNB-75	0.580	1.025	0.760	0.423	0.328	0.335	0.415	40	-27	-43	-42	-29	< 3.25E-9	>	1.29E-8	>	3.25E-5
U251	0.213	0.947	0.699	0.323	0.280	0.272	0.292	66	15	9	8	11	6.73E-9	>	3.25E-5	>	3.25E-5
Melanoma																	
LOX IMVI	0.254	1.351	0.862	0.555	0.538	0.561	0.686	55	27	26	28	39	5.08E-9	>	3.25E-5	>	3.25E-5
MAUVE-3M	0.450	0.730	0.661	0.516	0.535	0.538	0.603	75	23	30	31	55	.	>	3.25E-5	>	3.25E-5
M14	0.435	1.468	1.334	0.569	0.264	0.304	0.706	87	13	-39	-30	26	1.03E-8	.	.	>	3.25E-5
SK-MEL-2	0.569	1.343	1.148	0.587	0.525	0.536	0.703	75	2	-8	-6	17	7.15E-9	.	.	>	3.25E-5
SK-MEL-28	0.364	0.934	0.778	0.606	0.632	0.614	0.619	73	42	47	44	45	1.83E-8	>	3.25E-5	>	3.25E-5
SK-MEL-5	0.867	2.575	2.440	1.260	0.967	0.990	1.178	92	23	6	7	18	1.32E-8	>	3.25E-5	>	3.25E-5
UACC-257	0.549	1.115	0.966	0.745	0.748	0.745	0.747	74	35	35	35	35	1.31E-3	>	3.25E-5	>	3.25E-5
UACC-62	0.590	1.858	1.601	0.936	0.848	0.851	0.941	80	27	20	21	28	1.20E-8	>	3.25E-5	>	3.25E-5
Ovarian Cancer																	
OVCAR-3	0.303	0.803	0.642	0.253	0.225	0.225	0.195	68	-17	-26	-26	-36	5.28E-9	>	2.07E-8	>	3.25E-5
OVCAR-4	0.375	1.357	1.267	0.896	0.842	0.821	0.822	91	53	48	45	46	1.16E-7	>	3.25E-5	>	3.25E-5
OVCAR-5	0.545	1.382	1.267	0.773	0.705	0.676	0.660	86	27	19	16	14	1.34E-8	>	3.25E-5	>	3.25E-5
OVCAR-8	0.384	1.492	1.307	0.688	0.508	0.488	0.661	83	27	11	9	25	1.28E-8	>	3.25E-5	>	3.25E-5
SK-OV-3	0.527	1.007	0.871	0.589	0.496	0.502	0.536	72	13	-6	-5	2	7.57E-9	.	.	>	3.25E-5
Renal cancer																	

Figure 4A

736-0	0.741	2.178	2.072	1.672	1.001	0.873	1.314	93	65	18	9	40	6.73E-8	3.25E-5	>	3.25E-5	
A498	0.808	1.352	1.263	0.944	0.759	0.752	0.858	84	25	-6	-7	9	1.21E-8	.	>	3.25E-5	
ACHN	0.287	1.114	1.149	1.043	0.746	0.614	0.595	104	91	55	40	37	7.13E-7	>	3.25E-5	>	3.25E-5
CAKI-1	0.505	1.870	1.787	1.724	1.218	0.894	0.671	94	89	52	28	12	4.03E-7	>	3.25E-5	>	3.25E-5
RXF 393	0.432	0.700	0.610	0.412	0.293	0.285	0.307	66	-5	-32	-34	-29	5.54E-9	2.80E-8	>	3.25E-5	
SN12C	0.312	1.073	0.935	0.548	0.480	0.476	0.564	82	31	22	22	33	1.37E-8	>	3.25E-5	>	3.25E-5
TK-10	0.564	1.092	1.006	0.938	0.659	0.613	0.592	84	71	18	9	5	3.04E-3	>	3.25E-5	>	3.25E-5
UO-31	0.286	0.957	0.870	0.834	0.657	0.492	0.408	87	82	55	31	18	5.33E-7	>	3.25E-5	>	3.25E-5
Prostate Cancer																	
DU-145	0.239	0.817	0.768	0.197	0.147	0.124	0.130	92	-18	-38	-48	-46	7.79E-9	2.23E-8	>	3.25E-5	
Breast cancer																	
MCF7	0.244	1.177	0.603	0.352	0.338	0.332	0.337	38	12	10	9	10	< 3.25E-9	>	3.25E-5	>	3.25E-5
NCI/ADR-RES	0.498	1.599	1.570	1.588	1.322	1.109	0.331	97	99	75	55	-34	3.74E-6	1.37E-5	>	3.25E-5	
MDA-MB-231/ATCC	0.530	1.123	1.131	0.671	0.558	0.598	0.598	101	24	5	11	11	1.49E-8	>	3.25E-5	>	3.25E-5
HS 576T	0.561	0.978	0.777	0.509	0.504	0.434	0.379	52	-9	-10	23	-33	3.49E-9	2.29E-8	>	3.25E-5	
MDA-MR-435	0.483	1.897	0.783	0.137	0.166	0.204	0.388	21	-72	-66	-58	-20	< 3.25E-9	5.50E-9	.		
BT-549	0.882	1.497	1.294	0.993	0.872	0.825	0.757	67	18	-1	-6	-14	7.23E-9	282E-7	>	3.25E-5	
T-47D	0.542	1.137	0.992	0.673	0.660	0.641	0.717	76	22	20	17	29	9.76E-9	>	3.25E-5	>	3.25E-5
MDA-MB-468	0.580	1.187	0.968	0.460	0.459	0.459	0.407	64	-21	-21	-21	-30	4.75E-9	1.85E-8	>	3.25E-5	

Figure 4B

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro																
NSC D – 747908/1			Experiment ID : 0808NS92					Test Type: 08					Units: molar			
Report Date : 5 October 2008			Test date: 18 August 2008					QNS :					MC :			
COMI : T-M-233(75987)			Stain reagent: SRB Dual-Pass Related					SSPL 0X3W								
Concentration Log10																
Time		Mean optical densities						Percent Growth								
Panel / cell line	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	7.5	-6.5	5.5	-4.5	GI50	TGI	LC50	
Leukemia																
CCRF-CEM	0.221	1.127	1.011	0.288	0.274	0.275	0.259	87	7	6	6	4	9.50E-9	>	3.25E-5	> 3.25E-5
HL-60(TB)	0.702	2.368	2.316	0.630	0.576	0.588	0.613	97	-10	-18	-16	-13	8.90E-9		2.61 E-8	> 3.25E-5
K-562	0.148	1.177	0.891	0.242	0.207	0.191	0.177	72	9	6	4	3	7.31 E-9	>	3.25E-5	> 3.25E-5
MOLT-4	0.328	1.109	1.259	0.670	0.526	0.473	0.446	119	44	25	19	15	2.69E-8	>	3.25E-5	> 3.25E-5
RPMI-8226	0.349	1.043	0.603	0.333	0.336	0.323	0.308	37	-5	-4	-7	-12	< 3.25E-9		2.50E-8	> 3.25E-5
SR	0.224	0.457	0.384	0.233	0.219	0.219	0.203	69	4	-2	-2	-9	6.30E-9		1.40E-7	> 3.25E-5
Non-small cell lung cancer																
A549/ATCC	0.185	1.066	0.851	0.378	0.320	0.323	0.326	76	22	15	16	16	9.74E-9	>	3.25E-5	> 3.25E-5
EKVX	0.578	1.835	1.656	1.132	1.036	1.087	1.051	86	44	36	41	38	2.35E-3	>	3.25E-5	> 3.25E-5
HOF-62	0.861	1.601	1.527	1.336	1.100	1.088	1.069	90	64	32	31	28	9.05E-8	>	3.25E-5	> 3.25E-5
NCI-H226	0.835	1.644	1.556	1.401	1.251	1.285	1.311	89	70	51	30	59	> 3.25E-5	>	3.25E-5	> 3.25E-5
NCI-H23	0.711	1.766	1.644	0.987	0.937	1.022	1.109	88	26	21	29	38	1.34E-8	>	3.25E-5	> 3.25E-5
NCI-H322M	0.603	1.446	1.369	0.866	0.861	0.830	0.848	91	31	31	27	29	1.57E-8	>	3.25E-5	> 3.25E-5
NCI-H460	0.201	1.660	1.095	0.331	0.266	0.265	0.267	61	9	4	4	4	5.34E-9	>	3.25E-5	> 3.25E-5
NCI H522	0.423	1.445	1.183	0.516	0.446	0.462	0.466	75	9	2	4	4	7.74E-9	>	3.25E-5	> 3.25E-5
Colon Cancer																
COLO 205	0.302	0.862	0.753	0.218	0.173	0.152	0.161	80	-28	-43	-50	-47	6.21 E-9		1.80E-8	> 3.25E-5
HCC-2998	0.459	1.276	1.055	0.519	0.373	0.429	0.482	73	7	-19	-7	3	7.26E-9		.	> 3.25E-5
HCT-116	0.214	1.649	0.945	0.303	0.267	0.266	0.296	51	6	4	4	6	3.41 E-9	>	3.25E-5	> 3.25E-5
HCT-15	0.283	1.377	1.307	1.270	0.898	0.484	0.447	94	90	56	18	15	4.74E-7	>	3.25E-5	> 3.25E-5
Hi29	0.220	1.287	0.707	0.242	0.201	0.213	0.205	46	2	-9	-3	-7	< 3.25E-9		4.98E-8	> 3.25E-5
KM12	0.208	1.006	0.682	0.253	0.229	0.266	0.242	59	6	3	7	4	4.87E-9	>	3.25E-5	> 3.25E-5
SW-520	0.182	1.153	0.313	0.481	0.482	0.632	0.612	65	31	31	46	44	8.92E-9	>	3.25E-5	> 3.25E-5
CNS cancer																
SF-268	0.380	1.122	0.984	0.571	0.520	0.539	0.555	81	26	19	21	24	1.19E-5	>	3.25E-5	> 3.25E-5
SF-295	0.624	2.106	1.947	1.203	0.696	0.694	0.690	89	39	5	5	4	1.97E-8	>	3.25E-5	> 3.25E-5
SF-539	0.774	2.033	1.804	0.822	0.501	0.503	0.560	82	4	-35	-35	-26	8.31 E-9		4.07E-8	> 3.25E-5
SNB-19	0.638	1.593	1.476	1.023	0.824	0.850	0.854	88	40	19	22	23	2.03E-8	>	3.25E-5	> 3.25E-5
SNB-75	0.580	1.092	0.869	0.514	0.376	0.402	0.449	56	-11	-35	-31	-23	4.04E-9		2.21 E-8	> 3.25E-5
U251	0.213	1.032	0.325	0.369	0.327	0.335	0.336	75	19	14	15	15	9.05E-9	>	3.25E-5	> 3.25E-5
Melanoma																
LOX IMVI	0.254	1.460	0.915	0.602	0.584	0.676	0.729	55	29	27	35	39	4.97E-9	>	3.25E-5	> 3.25E-5
MAUVE-3M	0.450	0.712	0.649	0.501	0.516	0.600	0.632	76	20	25	57	70	.	>	3.25E-5	> 3.25E-5
M14	0.435	1.507	1.289	0.630	0.303	0.541	0.689	80	18	-30	10	24	9.87E-9		.	> 3.25E-5
SK-MEL-2	0.569	1.416	1.349	0.671	0.571	0.653	0.700	92	12	.	10	15	1.09E-8	>	325E-5	> 3.25E-5
SK-MEL-28	0.364	0.988	0.887	0.678	0.670	0.809	0.758	84	50	49	71	63	.	>	3.25E-5	> 3.25E-5
SK-MEL-5	0.867	2.659	2.677	1.385	0.968	1.073	1.158	101	29	6	11	16	1.66E-8	>	3.25E-5	> 3.25L-0
UACC-257	0.549	1.206	1.127	0.861	0.840	0.873	0.850	38	47	44	49	46	2.81 E-3	>	3.25E-5	> 3.25E-5
UACC-62	0.590	1.855	1.525	0.981	0.350	0.963	1.046	74	31	21	29	36	1.17E-8	>	325E-5	> 3.25E-5
Ovarian Cancer																
OVCAR-3	0.303	0.874	0.677	0.311	0.245	0.223	0.228	65	1	-19	-25	-25	5.65E-9		3.80E-8	> 3.25E-5
OVCAR-4	0.375	1.385	1.379	0.959	0.874	0.877	0.844	99	58	49	50	46	2.78E-7	>	3.25E-5	> 3.25E-5
OVCAR-5	0.545	1.379	1.324	0.697	0.651	0.666	0.725	93	18	13	14	22	1.23E-8	>	3.25E-5	> 3.25E-5
OVCAR-8	0.384	1.628	1.579	0.814	0.628	0.669	0.733	96	35	20	23	28	1.82E-8	>	325E-5	> 3.25E-5
SK-OV-3	0.527	1.064	0.977	0.664	0.534	0.549	0.524	84	25	1	4	-1	1.23E-8		2.36E-5	> 3.25E-5

Figure 5A

Renal cancer																
736-0	0.741	2.278	2.135	1.652	1.019	1.022	1.193	91	59	18	18	29	5.46E-8	>	3.25E-5	> 3.25E-5
A498	0.808	1.442	1.383	1.111	0.797	0.920	0.995	91	46	-1	16	29	2.88E-3	.	>	3.25E-5
ACHN	0.237	1.156	1.176	1.040	0.781	0.630	0.632	102	87	57	39	40	7.99E-7	>	3.25E-5	> 3.25E-5
CAKI-1	0.505	2.028	1.886	1.864	1.407	0.888	0.809	91	89	59	25	20	6.07E-7	>	3.25E-5	> 3.25E-5
RXF 393	0.432	0.759	0.695	0.459	0.317	0.339	0.369	80	8	-27	-22	-15	8.56E-9	.	5.55E-8	> 3.25E-5
SN12C	0.312	1.134	0.940	0.546	0.499	0.540	0.562	76	28	23	28	30	1.15E-8	>	3.25E-5	> 3.25E-5
TK-10	0.564	1.083	1.027	0.893	0.691	0.640	0.628	89	63	24	15	12	7.13E-3	>	3.25E-5	> 3.25E-5
UO-31	0.286	1.005	0.983	0.883	0.726	0.485	0.427	98	83	61	28	20	6.99E-7	>	3.25E-5	> 3.25E-5
Prostate Cancer																
DU-145	0.239	0.864	0.780	0.192	0.132	0.125	0.122	87	-20	-45	-48	-49	7.16E-9	.	2.11 E-8	> 3.25E-5
Breast cancer																
MCF7	0.244	1.279	0.693	0.359	0.344	0.355	0.331	43	11	10	11	8	<3.25E-9	>	3.25E-5	> 3.25E-5
NCI/ADR-RES	0.498	1.682	1.671	1.542	1.205	0.786	0.680	99	83	60	24	15	6.10E-7	>	3.25E-5	> 3.25E-5
MDA-MB-231	0.530	1.184	1.135	0.749	0.590	0.550	0.622	93	33	9	3	14	1.70E-8	>	3.25E-5	> 3.25E-5
/ATCC																
HS 576T	0.561	1.044	0.913	0.587	0.547	0.513	0.525	73	5	2	-9	-7	7.07E-9	.	1.55E-7	> 3.25E-5
MDA-MR-435	0.483	1.883	0.937	0.269	0.258	0.434	0.525	32	-44	-47	-10	3	<3.25E-9	.	>	3.25E-5
BT-549	0.832	1.349	1.165	0.837	0.771	0.708	0.759	61	-5	-13	-20	-14	4.72E-9	.	2.71 E-8	> 3.25E-5
T-47D	0.542	1.151	1.021	0.651	0.694	0.699	0.679	79	18	25	26	23	9.63E-9	>	3.25E-5	> 3.25E-5
MDA-MB-468	0.580	1.162	1.016	0.425	0.386	0.413	0.398	75	-27	-33	-29	-31	5.72E-9	.	1.77E-8	> 3.25E-5

Figure 5B

National Cancer Institute Developmental Therapeutics Program In-Vitro Test Results In-Vitro															
NSC D – 749484/1			Experiment ID: 0903NS66						Test Type: 08				Units: molar		
Report Date : 11 mapra 2010			Test date: 30 mapra 2009						QNS :				MC :		
COMI : T-M-220 (82279)			Stain reagent: SRB Dual-Pass Related						SSPL 0X3W						
Panel / cell line	Time		Concentration Log10						Percent Growth				GI50	TGI	LC50
	Zero	Control	-8.5	-7.5	6.5	-5.5	-4.5	-8.5	7.5	-6.5	5.5	-4.5			
Leukemia															
CCRF-CEM	0.646	1.658	1.565	0.853	0.821	0.896	0.825	91	20	17	25	18	1.90E-8	> 5.00E-5	> 5.00E-5
HL-60(TB)	0.592	1.750	1.072	0.467	0.429	0.458	0.486	41	-21	-28	-23	-18	< 5.00E-9	229E-8	> 5.00E-5
K-562	0.266	1.774	1.010	0.469	0.411	0.422	0.422	49	13	10	10	10	<, 5.00E-9	> 5.00E-5	> 5.00E-5
MOLT-4	0.667	1.914	1.637	1.014	0.968	1.219	0.93	78	28	24	44	22	1.80E-8	> 5.00E-5	> 5.00E-5
6															
RPMI-8226	0.636	1.747	1.039	0.736	0.752	0.728	0.729	36	14	10	8	8	< 5.00E-9	> 5.00E-5	> 5.00E-5
SR	0.262	0.675	0.369	0.283	0.274	0.275	0.243	26	5	3	3	-7	< 5.00E-9	9.73E-6	> 5.00E-5
Non-small cell lung cancer															
A549/ATCC	0.291	1.490	1.006	0.770	0.709	0.849	0.785	60	40	35	47	4	1.54E-8	> 5.00E-5	> 5.00E-5
EKVX	0.353	1.415	1.085	0.783	0.788	0.657	0.833	69	40	41	47	45	2.31 E-8	> 5.00E-5	> 5.00E-5
HOF-62	0.509	1.394	1.192	0.922	0.792	0.908	0.838	77	47	32	45	37	3.88E-8	> 5.00E-5	> 5.00E-5
HOP-92	0.872	1.341	1.212	1.177	1.160	1.170	1.054	72	65	61	64	39	1.76E-5	> 5.00E-5	> 5.00E-5
NCI-H226	0.580	1.379	1.202	1.058	0.938	1.040	0.871	78	60	45	58	36	.	> 5.00E-5	> 5.00E-5
NCI-H23	0.323	1.135	0.824	0.578	0.505	0.670	0.641	62	31	22	43	39	1.21E-8	> 5.00E-5	> 5.00E-5
NCI-H322M	0.508	1.339	1.036	0.733	0.712	0.741	0.900	64	27	25	28	47	1.18E-3	> 5.00E-5	> 5.00E-5
NCI-H460	0.203	1.881	0.703	0.386	0.345	0.412	0.348	30	11	8	12	9	< 5.00E-9	> 5.00E-5	> 5.00E-5
NCI H522	0.606	1.530	1.149	0.779	0.745	0.896	0.849	59	19	15	31	26	8.27E-9	> 5.00E-5	> 5.00E-5
Colon Cancer															
COLO 205	0.294	0.968	0.442	0.211	0.194	0.220	0.185	22	-28	-34	-25	-37	< 5.00E-9	1.36E-8	> 5.00E-5
HCC-2998	1.219	2.929	2.623	1.222	0.879	0.985	0.987	82	-	-28	-19	-19	1.23E-8	5.06E-8	> 5.00E-5
HCT-116	0.188	1.370	0.504	0.348	0.254	0.436	0.411	27	13	6	21	19	< 5.00E-9	> 5.00E-5	> 5.00E-5
HCT-15	0.469	2.275	2.271	1.965	1.135	0.849	0.821	100	83	37	21	19	2.59E-7	> 5.00E-5	> 5.00E-5
HI29	0.210	1.369	0.610	0.293	0.284	0.303	0.270	34	7	6	8	5	< 5.00E-9	> 5.00E-5	> 5.00E-5
KM12	0.188	0.740	0.301	0.199	0.188	0.253	0.243	20	2	.	12	10	< 5.00E-9	.	> 5.00E-5
SW-520	0.213	1.244	0.806	0.631	0.656	0.786	0.660	57	41	43	56	43	.	> 5.00E-5	> 5.00E-5
CNS cancer															
SF-268	0.397	1.046	0.805	0.594	0.530	0.678	0.596	63	30	21	43	31	1.25E-8	> 5.00E-5	> 5.00E-5
SF-295	0.731	2.771	2.348	1.527	1.072	1.164	1.282	79	39	17	71	27	2.67E-8	5.00E-5	> 5.00E-5
SNB-19	0.717	1.386	1.301	1.226	1.173	1.300	1.246	87	76	68	87	79	> 5.00E-5	5.00E-5	> 5.00E-5
SNB-75	0.557	1.255	0.901	0.602	0.551	0.690	0.641	49	6	-1	19	12	< 5.00E-9	.	> 5.00E-5
U251	0.288	1.347	0.866	0.587	0.536	0.602	0.483	55	28	23	30	18	7.45E-9	> 5.00E-5	> 5.00E-5
Melanoma															
LOX IMVI	0.274	1.563	1.027	0.951	0.911	1.014	0.824	58	52	49	57	43	.	> 5.00E-5	> 5.00E-5
MAUVE-3M	0.556	1.091	0.901	0.867	0.910	0.985	0.986	64	58	66	80	60	> 5.00E-5	> 5.00E-5	> 5.00E-5
M14	0.243	0.942	0.635	0.287	0.248	0.475	0.447	56	6	1	33	29	6.62E-9	> 5.00E-5	> 5.00E-5
MDA-MB-435	0.372	2.112	0.640	0.378	0.529	0.617	0.662	15	.	9	14	17	< 5.00E-9	> 5.00E-5	> 5.00E-5
SK-MEL-2	0.652	1.553	1.421	0.969	0.912	1.023	0.852	85	35	29	41	22	2.53E-8	> 5.00E-5	> 5.00E-5
SK-MEL-28	0.615	1.531	1.305	1.236	1.219	1.274	1.077	75	68	66	72	50	> 5.00E-5	> 5.00E-5	> 5.00E-5
SK-MEL-5	0.345	2.011	1.045	0.636	0.750	0.786	0.571	42	20	24	26	14	< 5.00E-9	> 5.00E-5	> 5.00E-5
UACC-257	0.681	1.564	1.488	1.416	1.369	1.424	1.332	91	83	78	84	74	> 5.00E-5	> 5.00E-5	> 5.00E-5
UACC-62	0.559	2.150	1.576	1.036	0.905	1.122	1.074	64	30	22	35	32	1.29E-8	> 5.00E-5	> 5.00E-5
Ovarian Cancer															
IGROV1	0.511	1.372	1.134	0.992	0.943	0.952	0.779	72	56	50	51	31	5.76E-6	> 5.00E-5	> 5.00E-5
OVCAR-3	0.362	0.832	0.566	0.273	0.244	0.253	0.246	43	-25	-33	-30	-32	< 5.00E-9	2.17E-8	> 5.00E-5
OVCAR-4	0.344	1.087	0.924	0.694	0.672	0.726	0.665	78	47	44	51	43	.	> 5.00E-5	> 5.00E-5

Figure 6A

OVCAR-5	0.361	0.948	0.776	0.556	0.518	0.582	0.525	71	33	27	38	28	1.78E-8	> 5.00E-5	> 5.00E-5
OVCAR-8	0.464	1.593	1.283	0.958	0.925	1.019	1.021	73	44	41	49	49	3.04E-8	> 5.00E-5	> 5.00E-5
NCI/ADR-RES	0.511	1.718	1.729	1.616	1.192	0.601	0.486	101	92	56	7	-5	6.77E-7	1.99E-5	> 5.00E-5
SK-OV-3	0.475	0.964	0.775	0.612	0.534	0.594	0.603	61	28	12	24	26	1.10E-8	> 5.00E-5	> 5.00E-5
Renal cancer															
786-O	0.584	1.989	1.754	1.402	1.001	1.438	1.428	83	58	30	61	60	.	> 5.00E-5	> 5.00E-5
A498	1.019	1.608	1.488	1.274	1.199	1.295	1.307	80	43	31	47	49	3.28E-3	> 5.00E-5	> 5.00E-5
ACHN	0.292	1.210	1.180	0.930	0.751	0.785	0.747	97	75	50	54	50	.	> 5.00E-5	> 5.00E-5
CAKI-1	0.612	2.918	2.734	2.146	1.594	1.397	1.439	92	67	43	34	36	2.45E-7	> 5.00E-5	> 5.00E-5
RXF 393	0.326	0.799	0.644	0.539	0.464	0.533	0.507	67	45	29	44	38	3.00E-8	> 5.00E-5	> 5.00E-5
SN12C	0.403	1.488	1.143	0.891	0.830	1.022	0.940	68	45	39	57	50	.	> 5.00E-5	> 5.00E-5
TK-10	0.608	1.387	1.249	0.933	0.855	0.901	0.859	82	48	32	38	32	4.41 E-8	> 5.00E-5	> 5.00E-5
UO-31	0.738	1.508	1.430	1.324	1.145	1.098	0.946	90	76	53	47	27	1.49E-6	> 5.00E-5	> 5.00E-5
Prostate Cancer															
PC-3	0.258	0.979	0.632	0.468	0.449	0.476	0.445	52	29	26	30	26	6.02E-9	> 5.00E-5	> 5.00E-5
DU-145	0.454	1.307	1.017	0.409	0.263	0.295	0.329	66	-10	-42	-35	-28	8.10E-9	3.69E-8	> 5.00E-5
Breast cancer															
MCF7	0.305	1.641	0.755	0.506	0.471	0.488	0.473	34	15	12	14	13	< 5.00E-9	> 5.00E-5	> 5.00E-5
MDA-MB-231	0.450	1.130	1.070	0.755	0.667	0.778	0.664	91	45	32	48	31	3.85E-3	> 5.00E-5	> 5.00E-5
/ATCC															
HS 578T	0.493	0.944	0.691	0.448	0.378	0.458	0.504	44	-9	-23	-7	2	< 5.00E-9	.	> 5.00E-5
BT-549	1.094	2.240	1.953	1.273	1.252	1.588	1.449	75	16	14	-43	31	1.32E-8	> 5.00E-5	> 5.00E-5
T-47D	0.445	0.931	0.674	0.568	0.526	0.615	0.616	47	25	17	35	35	< 5.00E-9	> 5.00E-5	> 5.00E-5
MDA-MB-468	0.521	1.402	1.065	0.658	0.679	0.771	0.713	62	16	18	28	22	8.98E-9	> 5.00E-5	> 5.00E-5

Figure 6B

Chemical	MW	Pharmacology original screened model	Inhibition (%)						IC ₅₀ (μM)
			0.0001 μM	0.001 μM	0.01 μM	0.1 μM	1 μM	1.0 μM	
Taxol	853	Normal cell HELF	10.96	15.32	12.56	42.15	56.96	71.09	0.5701
		Anti-colon cancer HCT-8	13.58	10.33	19.75	34.03	50.70	64.98	1.1030
		Anti-liver cancer BEL-7402	5.18	6.09	9.17	53.60	70.04	73.57	0.307
		Anti-ovarian cancer A278	11.14	15.57	9.68	36.92	69.85	66.15	0.4855
		Anti-lung Cancer A549	3.83	14.47	35.92	62.02	63.86	58.90	0.0573
		Anti-gastric cancer BGC-823	7.51	13.92	78.63	86.40	85.49	79.33	0.0029
		Anti-breast Cancer MCF-7	13.38	13.11	21.37	51.88	69.81	70.95	0.2219
		Anti-cervical cancerHeLa	-12.33	25.81	72.40	81.18	79.66	72.40	0.0016
		Anti-nasopharyngeal cancer KB	4.19	64.75	87.98	96.19	90.86	94.56	0.0005
MTC-301	1425	Anti-kidney cancer KeTr3	17.62	19.09	18.22	59.54	62.63	79.15	0.1900
		Normal cell HELF	7.08	12.30	15.08	25.41	41.57	63.42	2.9240
		Anti-colon cancer HCT-8	10.26	12.38	9.52	20.38	32.41	61.51	5.3620
		Anti-liver cancer BEL-7402	1.76	4.93	7.37	18.14	63.15	75.37	0.6529
		Anti-ovarian cancer A278	8.61	10.49	13.73	24.13	45.11	58.66	3.6750
		Anti-lung Cancer A549	-3.38	2.86	8.21	44.31	68.34	66.97	0.1988
		Anti-gastric cancer BGC-823	7.27	12.43	40.41	83.14	83.62	82.82	0.0120
		Anti-breast Cancer MCF-7	-4.52	4.99	10.60	27.18	56.53	69.28	0.8325
		Anti-cervical cancerHeLa	-3.31	-24.81	17.88	60.61	69.82	65.73	0.0270
MTC-302	1423	Anti-nasopharyngeal cancer KB	7.02	2.14	30.48	89.39	88.35	99.84	0.0176
		Anti-kidney cancer KeTr3	13.90	9.27	4.23	20.65	58.12	70.65	1.1810
		Normal cell HELF	-1.43	4.96	-2.35	20.42	56.32	64.02	1.1080
		Anti-colon cancer HCT-8	-1.40	-4.56	7.20	20.24	50.14	60.33	1.4640
		Anti-liver cancer BEL-7402	1.59	-4.82	1.18	29.47	70.39	75.52	0.2855
		Anti-ovarian cancer A278	5.33	3.35	2.65	18.10	63.15	65.66	0.8811
		Anti-lung Cancer A549	-0.90	-7.19	21.83	47.93	64.49	69.55	0.1457
		Anti-gastric cancer BGC-823	-0.30	-0.55	66.73	76.74	72.28	79.30	0.0054
		Anti-breast Cancer MCF-7	1.20	-10.58	5.48	29.59	59.06	64.59	0.7297
MTC-303	1445	Anti-cervical cancerHeLa	-9.32	-17.91	60.51	70.38	76.69	75.43	0.0081
		Anti-nasopharyngeal cancer KB	-7.52	-0.28	51.84	77.35	75.41	78.74	0.0064
		Anti-kidney cancer KeTr3	8.79	10.46	8.56	43.02	61.42	79.96	0.3894
		Normal cell HELF	3.38	1.11	-1.64	21.71	64.39	64.52	0.5706
		Anti-colon cancer HCT-8	5.21	3.23	-1.13	9.51	49.35	62.23	2.1110
		Anti-liver cancer BEL-7402	-4.00	1.09	-5.44	46.42	83.73	79.22	0.0978
		Anti-ovarian cancer A278	16.63	17.42	5.51	24.47	63.51	61.56	1.2630
		Anti-lung Cancer A549	20.00	19.98	36.90	56.73	69.47	70.21	0.0589
		Anti-gastric cancer BGC-823	9.16	15.34	77.56	80.50	91.07	87.28	0.0037
MTC-304	1461	Anti-breast Cancer MCF-7	-8.18	-6.20	3.90	23.57	55.58	57.46	0.8809
		Anti-cervical cancerHeLa	5.92	3.90	79.38	87.62	95.47	92.25	0.0043
		Anti-nasopharyngeal cancer KB	14.84	17.93	40.89	87.52	94.26	95.88	0.0113
		Anti-kidney cancer KeTr3	33.86	-7.74	19.81	51.53	81.49	78.93	0.0976
		Normal cell HELF	-2.72	-2.99	4.69	16.13	64.68	62.63	0.7312
		Anti-colon cancer HCT-8	4.08	3.81	10.54	17.50	46.99	72.56	1.5500
		Anti-liver cancer BEL-7402	-2.46	-5.42	-12.84	22.51	75.36	84.16	0.2627
		Anti-ovarian cancer A278	2.85	19.62	11.25	22.00	56.27	66.18	1.1880
		Anti-lung Cancer A549	13.33	22.42	27.50	58.01	71.42	63.35	0.0782
		Anti-gastric cancer BGC-823	10.32	17.89	86.82	90.49	94.66	96.46	0.0031
		Anti-breast Cancer MCF-7	-11.05	-3.03	4.54	16.52	57.44	56.65	1.0080
		Anti-cervical cancerHeLa	10.14	9.73	58.63	85.16	86.69	88.81	0.0060
		Anti-nasopharyngeal cancer KB	-5.15	17.07	23.45	78.49	89.66	97.54	0.0281
		Anti-kidney cancer KeTr3	6.95	24.81	16.39	37.61	70.10	72.77	0.3392

Figure 7

Chemical	MW	Pharmacology original screened model	Inhibition (%)						IC ₅₀ (μM)
			0.0001 μM	0.001 μM	0.01 μM	0.1 μM	1 μM	1.0 μM	
MTC-305	1461	Normal cell HELF	-0.29	-12.62	2.24	18.78	57.59	59.44	1.000
		Anti-colon cancer HCT-8	6.02	5.41	11.13	23.62	48.55	57.87	2.3870
		Anti-liver cancer BEL-7402	-6.45	-5.79	-8.87	26.41	83.17	82.88	0.1811
		Anti-ovarian cancer A278	11.39	7.89	9.76	17.55	49.16	58.65	2.8580
		Anti-lung Cancer A549	15.61	-7.70	28.15	40.56	52.36	59.55	0.8078
		Anti-gastric cancer BGC-823	6.80	14.41	75.58	81.22	82.47	81.35	0.0035
		Anti-breast Cancer MCF-7	2.06	-12.59	3.72	22.75	56.91	56.04	1.0720
		Anti-cervical cancerHeLa	11.09	-20.79	57.51	73.79	73.18	79.30	0.0088
		Anti-nasopharyngeal cancer KB	1.69	6.24	49.50	89.67	85.94	94.11	0.0092
MTC-306	1427	Anti-kidney cancer KeTr3	8.41	-14.03	8.36	35.34	70.78	70.99	0.2333
		Normal cell HELF	-14.8	-11.4	-7.1	22.9	65.3	63.3	0.3940
		Anti-colon cancer HCT-8	-6.8	-9.1	1.9	3.4	47.2	54.8	2.4380
		Anti-liver cancer BEL-7402	10.7	10.1	6.2	28.5	78.0	79.2	0.2573
		Anti-ovarian cancer A278	-3.8	8.7	11.5	38.1	59.4	78.6	0.3126
		Anti-lung Cancer A549	3.7	-4.6	35.7	60.1	59.7	59.7	0.1190
		Anti-gastric cancer BGC-823	9.9	15.4	61.9	80.1	75.8	81.2	0.0046
		Anti-breast Cancer MCF-7	6.1	18.6	19.7	42.6	55.7	65.8	0.7353
		Anti-cervical cancerHeLa	3.9	30.5	87.3	94.8	93.3	94.4	0.0017
MTC-307	1427	Anti-nasopharyngeal cancer KB	2.8	28.9	79.6	88.8	83.9	91.9	0.0020
		Anti-kidney cancer KeTr3	-0.3	9.2	22.1	72.4	87.6	89.2	0.0367
		Normal cell HELF	-26.4	-8.7	-19.8	15.9	61.3	68.4	0.4437
		Anti-colon cancer HCT-8	-18.2	-28.1	-0.6	7.8	48.4	55.3	1.2680
		Anti-liver cancer BEL-7402	-3.2	1.7	3.8	16.8	82.5	83.2	0.2712
		Anti-ovarian cancer A278	-17.9	5.5	-0.8	31.6	63.6	64.4	0.3482
		Anti-lung Cancer A549	2.3	-11.9	22.4	43.0	64.5	50.5	0.4811
		Anti-gastric cancer BGC-823	9.5	8.9	46.9	71.1	72.8	79.2	0.0035
		Anti-breast Cancer MCF-7	-5.2	6.9	64.9	26.8	58.3	66.6	0.8300
MTC-308	1445	Anti-cervical cancerHeLa	-9.9	12.3	63.9	86.0	91.6	89.6	0.0040
		Anti-nasopharyngeal cancer KB	-2.4	16.3	63.9	84.0	84.1	88.0	0.0036
		Anti-kidney cancer KeTr3	-8.0	1.1	-14.0	54.1	78.4	96.7	0.0930
		Normal cell HELF	-11.7	-14.4	-5.3	7.6	48.7	51.6	2.2770
		Anti-colon cancer HCT-8	-3.8	-12.7	0.7	-10.7	37.9	50.8	4.6000
		Anti-liver cancer BEL-7402	-2.8	-13.0	-9.2	3.2	75.0	78.0	0.3802
		Anti-ovarian cancer A278	3.9	6.8	13.7	28.1	67.5	69.8	0.4262
		Anti-lung Cancer A549	-0.6	-9.2	27.2	33.4	45.8	47.9	IC ₅₀ >10
		Anti-gastric cancer BGC-823	5.7	19.6	64.4	67.2	68.6	74.2	0.0030
MTC-308	1445	Anti-breast Cancer MCF-7	-3.8	-1.8	13.2	30.2	39.1	57.7	2.8470
		Anti-cervical cancerHeLa	11.7	7.2	74.9	91.7	85.4	92.0	0.0048
		Anti-nasopharyngeal cancer KB	12.4	7.4	69.6	81.7	81.7	85.5	0.0056
		Anti-kidney cancer KeTr3	13.7	10.7	12.2	52.5	72.3	84.0	0.1674
		Normal cell HELF	-11.7	-14.4	-5.3	7.6	48.7	51.6	2.2770

Figure 8

Chemical	MW	Pharmacology original screened model	Inhibition (%)						IC ₅₀ (μM)
			0.0001 μM	0.001 μM	0.01 μM	0.1 μM	1 μM	1.0 μM	
Docetaxel	807.0	Normal cell HELF	11.34	34.32	41.71	58.04	52.13	95.04	0.240
		Anti-colon cancer HCT-8	8.71	21.64	42.36	47.20	50.81	90.76	0.664
		Anti-liver cancer BEL-7402	7.90	23.85	35.01	48.61	43.76	86.10	0.859
		Anti-ovarian cancer A278	6.56	22.47	14.51	42.21	38.95	90.52	2.724
		Anti-lung Cancer A549	28.32	33.98	35.83	38.56	28.29	84.61	28.050
		Anti-gastric cancer BGC-823	93.44	83.33	82.50	80.55	77.29	85.99	0<IC ₅₀ <0.001
		Anti-breast Cancer MCF-7	26.93	34.07	43.06	48.19	45.62	82.02	0.609
		Anti-cervical cancer HeLa	75.05	79.34	83.76	83.62	82.65	93.87	0<IC ₅₀ <0.001
		Anti-nasopharyngeal cancer KB	8272	85.99	88.43	87.03	88.38	97.63	0<IC ₅₀ <0.001
MDC-403	1399.0	Anti-kidney cancer KeTr3	17.19	48.71	74.79	75.79	74.91	84.79	0.006
		Normal cell HELF	7.32	25.92	51.61	58.04	61.98	96.85	0.161
		Anti-colon cancer HCT-8	16.01	15.60	39.00	53.38	56.96	87.29	0.577
		Anti-liver cancer BEL-7402	7.92	14.14	33.16	51.96	46.22	69.87	1.288
		Anti-ovarian cancer A278	2.85	11.47	29.03	37.60	38.95	69.11	8.643
		Anti-lung Cancer A549	32.59	28.56	41.29	32.41	30.54	73.49	95.930
		Anti-gastric cancer BGC-823	69.26	83.33	82.90	81.30	78.98	98.06	0<IC ₅₀ <0.001
		Anti-breast Cancer MCF-7	25.01	27.35	41.29	45.34	48.65	67.71	2.808
		Anti-cervical cancer HeLa	50.77	62.29	76.50	74.70	77.80	96.80	0<IC ₅₀ <0.001
MDC404	1315.0	Anti-nasopharyngeal cancer KB	70.63	82.85	84.17	83.86	84.28	98.52	0<IC ₅₀ <0.001
		Anti-kidney cancer KeTr3	8.27	24.81	69.82	74.49	72.04	98.56	0.031
		Normal cell HELF	3.47	4.63	51.17	62.82	66.11	92.81	0.185
		Anti-colon cancer HCT-8	8.33	4.42	24.26	41.13	57.04	79.28	2.355
		Anti-liver cancer BEL-7402	-2.52	1.51	26.72	43.64	48.89	68.72	2.258
		Anti-ovarian cancer A278	-4.35	2.83	27.70	40.76	44.56	73.01	3.858
		Anti-lung Cancer A549	27.12	23.80	36.41	36.24	29.66	71.68	0<IC ₅₀ <0.001
		Anti-gastric cancer BGC-823	55.48	79.41	83.59	82.07	81.90	99.46	0<IC ₅₀ <0.001
		Anti-breast Cancer MCF-7	18.13	20.70	37.12	43.79	43.03	66.52	8.303
MDC405	1315.0	Anti-cervical cancer HeLa	30.20	55.62	77.47	76.54	74.46	94.70	0.002
		Anti-nasopharyngeal cancer KB	60.16	76.01	83.99	83.91	89.86	98.28	0<IC ₅₀ <0.001
		Anti-kidney cancer KeTr3	-1.97	10.63	69.72	74.77	75.35	99.36	0.036
		Normal cell HELF	6.97	27.23	43.85	56.35	54.30	92.19	0.522
		Anti-colon cancer HCT-8	5.08	11.58	44.60	66.29	65.48	77.74	0.265
		Anti-liver cancer BEL-7402	7.00	19.93	37.71	37.27	35.21	69.95	23.020
		Anti-ovarian cancer A278	-6.27	11.22	27.52	45.13	42.45	73.99	3.993
		Anti-lung Cancer A549	32.34	36.45	36.83	35.72	29.68	70.47	61.140
		Anti-gastric cancer BGC-823	13.03	51.16	73.09	73.70	73.60	82.04	0.007
		Anti-breast Cancer MCF-7	18.16	26.26	39.62	46.39	38.88	59.38	47.270
		Anti-cervical cancer HeLa	82.48	85.63	88.24	85.03	87.97	96.00	0<IC ₅₀ <0.001
		Anti-nasopharyngeal cancer KB	67.06	79.57	81.75	86.16	87.71	98.48	0<IC ₅₀ <0.001
		Anti-kidney cancer KeTr3	6.00	28.22	68.28	73.41	71.72	98.42	0.037

Figure 9

Chemical	MW	Pharmacology original screened model	Inhibition (%)						IC ₅₀ (μM)
			0.0001 μM	0.001 μM	0.01 μM	0.1 μM	1 μM	10 μM	
MDC-406	1381.0	Normal cell HELF	1.76	19.02	36.62	52.03	53.52	81.61	1.142
		Anti-colon cancer HCT-8	8.64	15.40	37.32	69.55	64.03	76.41	0.352
		Anti-liver cancer BEL-7402	0.61	22.26	36.91	43.30	39.75	62.97	6.561
		Anti-ovarian cancer A278	-2.13	7.08	28.91	41.90	39.99	69.92	6.739
		Anti-lung Cancer A549	34.82	33.73	35.38	30.47	32.03	69.34	>100
		Anti-gastric cancer BGC-823	46.61	52.05	73.51	73.60	70.86	76.45	0.001<IC ₅₀ <0.01
		Anti-breast Cancer MCF-7	20.02	26.19	38.95	45.59	44.49	67.11	38.200
		Anti-cervical cancerHeLa	82.16	84.01	86.73	84.10	84.24	97.24	0<IC ₅₀ <0.001
		Anti-nasopharyngeal cancer KB	70.63	78.00	80.85	78.35	82.58	99.18	0<IC ₅₀ <0.001
MDC-407	1381.0	Anti-kidney cancer KeTr3	4.19	21.51	65.00	71.25	70.00	95.30	0.054
		Normal cell HELF	13.59	23.10	45.14	54.98	58.08	74.00	0.649
		Anti-colon cancer HCT-8	9.44	13.38	40.84	60.24	66.06	82.28	0.500
		Anti-liver cancer BEL-7402	2.35	13.41	34.47	48.80	39.91	70.11	2.386
		Anti-ovarian cancer A278	1.84	8.02	27.16	45.25	41.90	79.73	4.290
		Anti-lung Cancer A549	32.86	33.81	33.39	32.87	35.63	56.70	>100
		Anti-gastric cancer BGC-823	68.51	65.70	71.29	73.70	74.26	80.08	0<IC ₅₀ <0.001
		Anti-breast Cancer MCF-7	23.85	26.70	41.91	45.27	45.06	54.32	35.620
		Anti-cervical cancerHeLa	76.33	83.99	86.38	85.78	73.46	96.53	0<IC ₅₀ <0.001
MDC-408	1399.0	Anti-nasopharyngeal cancer KB	71.15	75.84	81.71	82.01	85.08	98.14	0<IC ₅₀ <0.001
		Anti-kidney cancer KeTr3	5.99	17.32	65.58	73.02	73.93	97.38	0.064
		Normal cell HELF	1.5	10.1	46.2	61.3	68.4	79.6	0.226
		Anti-colon cancer HCT-8	5.3	9.6	39.2	48.2	73.7	72.0	0.609
		Anti-liver cancer BEL-7402	0.8	12.5	30.4	43.9	53.1	72.8	2.458
		Anti-ovarian cancer A278	-1.7	12.3	38.7	52.2	57.0	68.9	1.048
		Anti-lung Cancer A549	22.8	36.9	46.9	51.3	50.1	75.7	0.402
		Anti-gastric cancer BGC-823	19.0	68.5	69.6	75.6	76.6	94.3	0.003
		Anti-breast Cancer MCF-7	11.5	23.3	42.2	54.4	54.0	57.7	2.756
		Anti-cervical cancerHeLa	44.2	80.2	81.2	81.0	86.4	88.3	0.001<IC ₅₀ <0.01
		Anti-nasopharyngeal cancer KB	19.4	64.9	80.5	86.3	86.1	97.2	0.004
		Anti-kidney cancer KeTr3	7.5	20.6	60.5	74.2	84.1	95.8	0.059

Figure 10

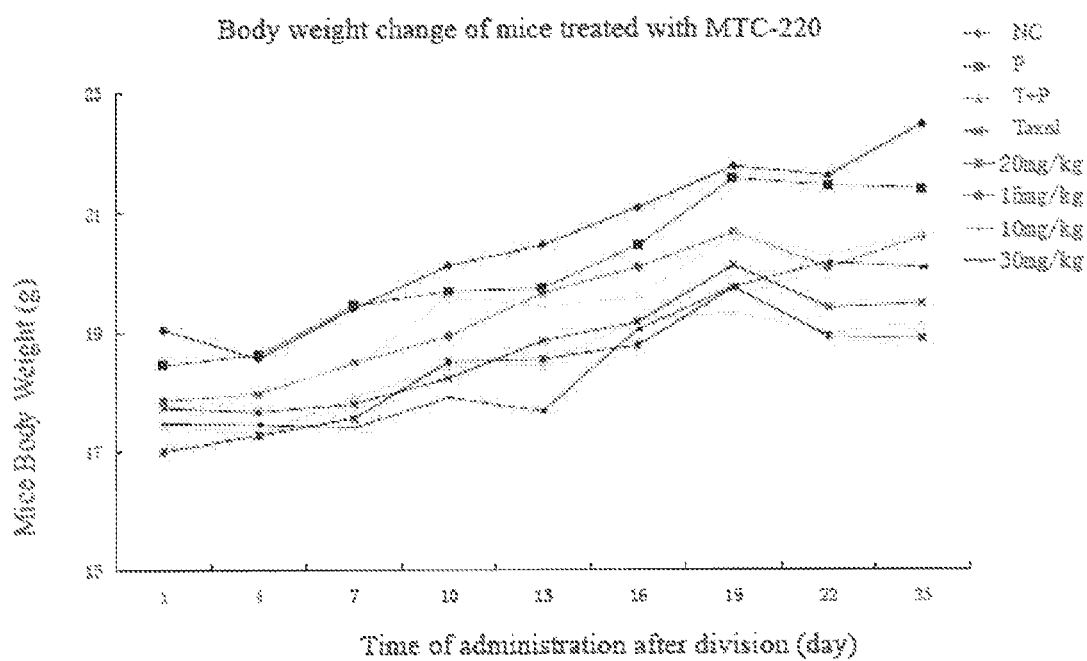


Figure 11

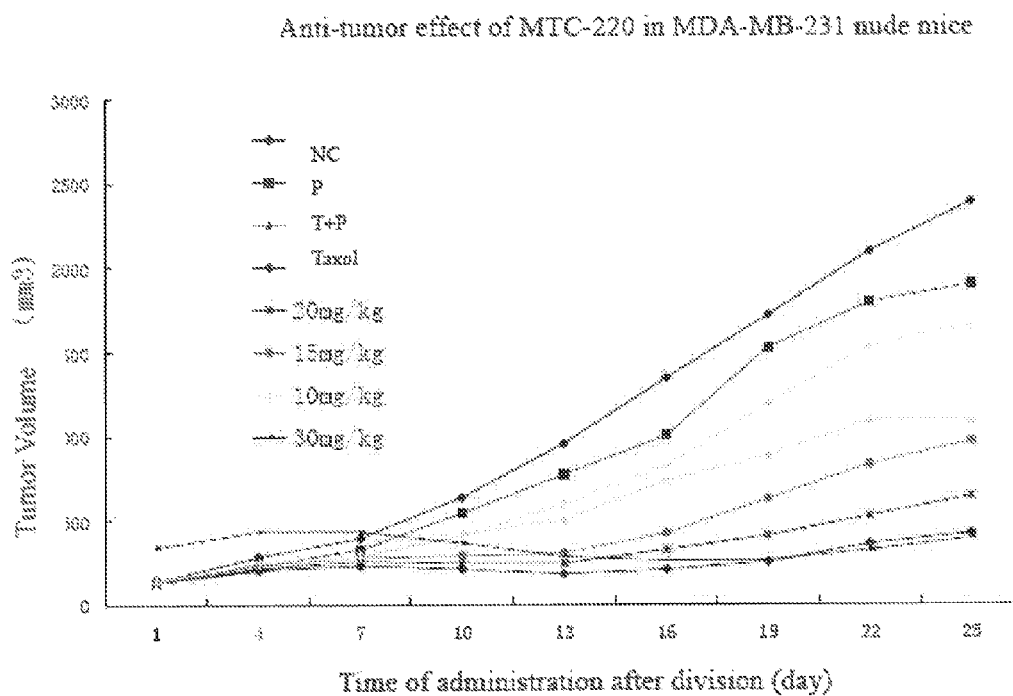


Figure 12

RTV change of treated with MTC-220 of similar dose in different administration method

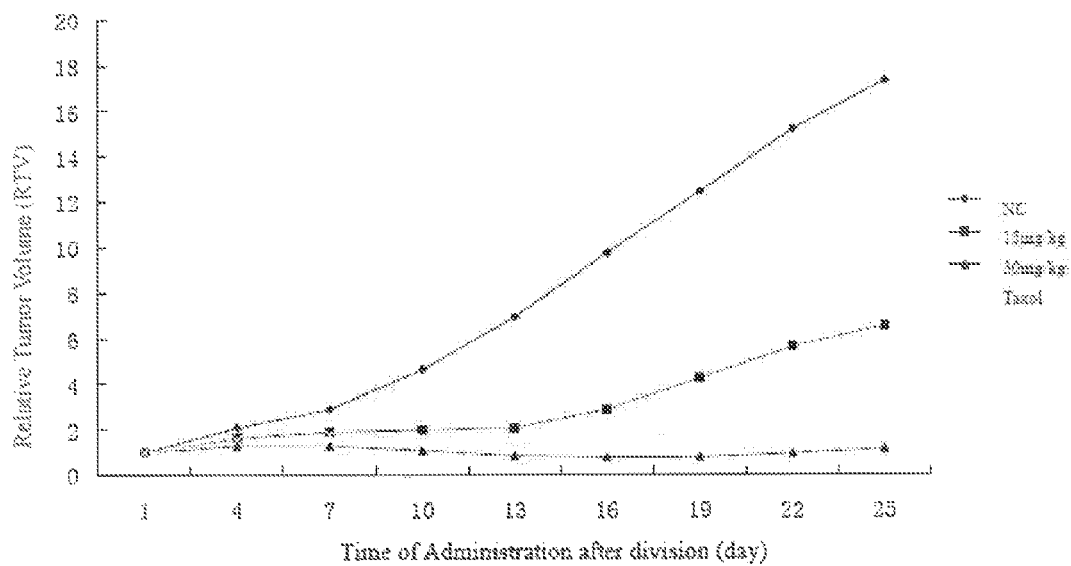


Figure 13

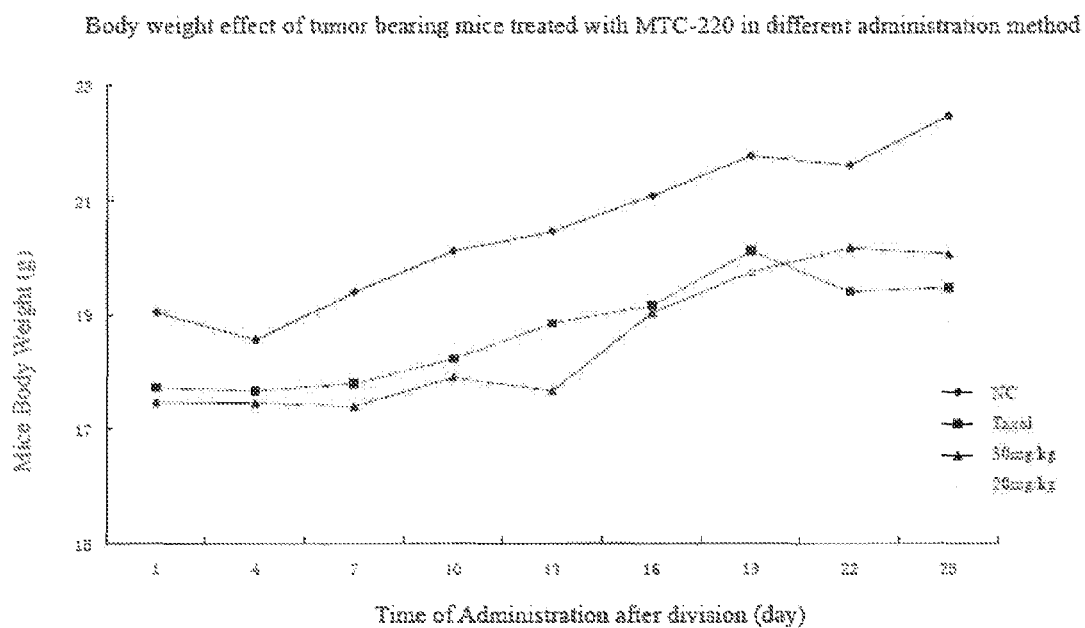


Figure 14

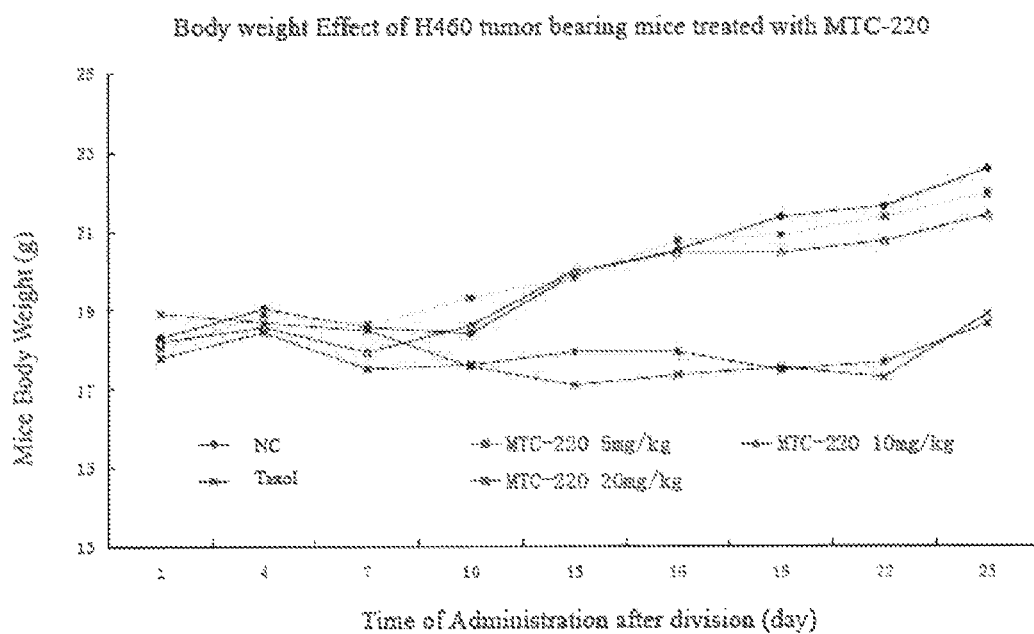
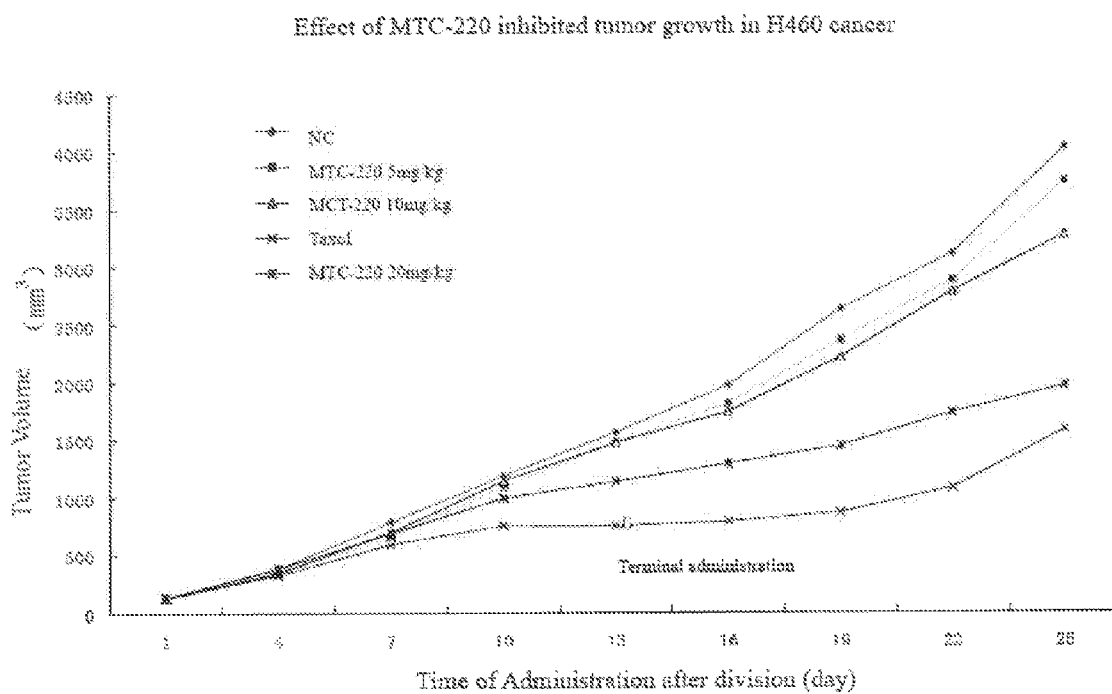
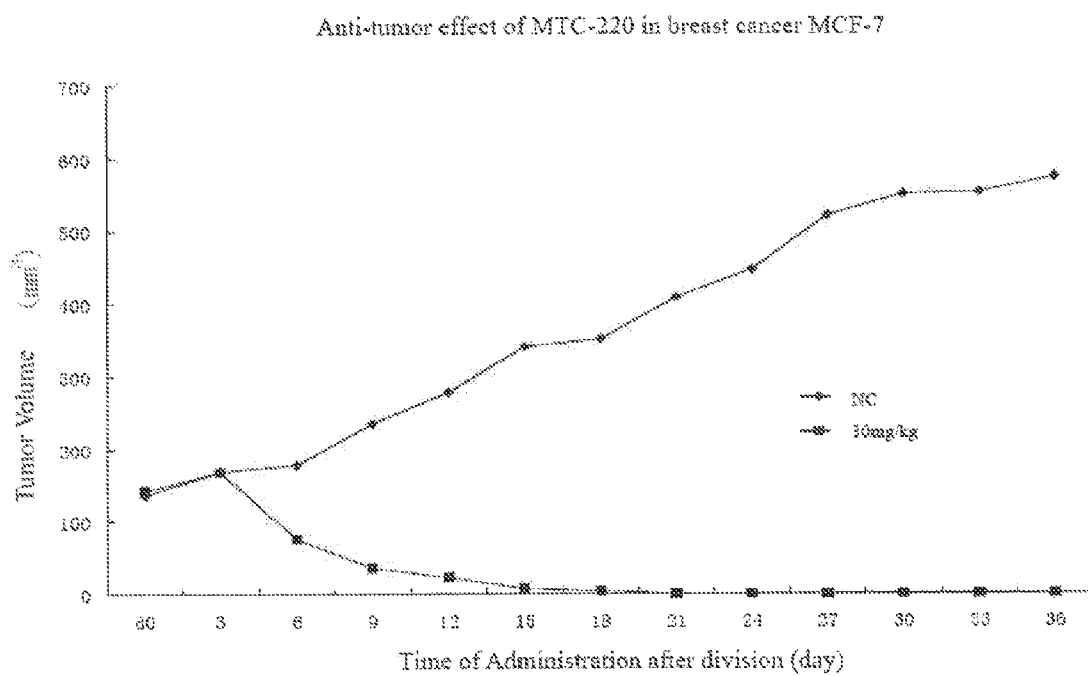
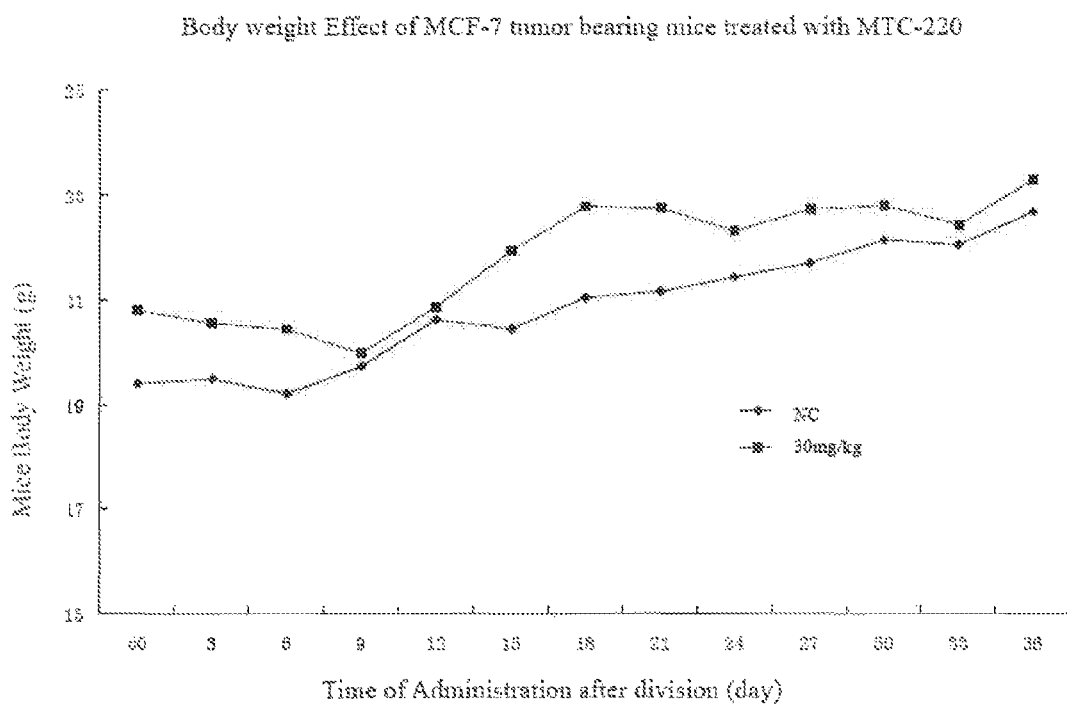


Figure 15

**Figure 16**

**Figure 17**

**Figure 18**

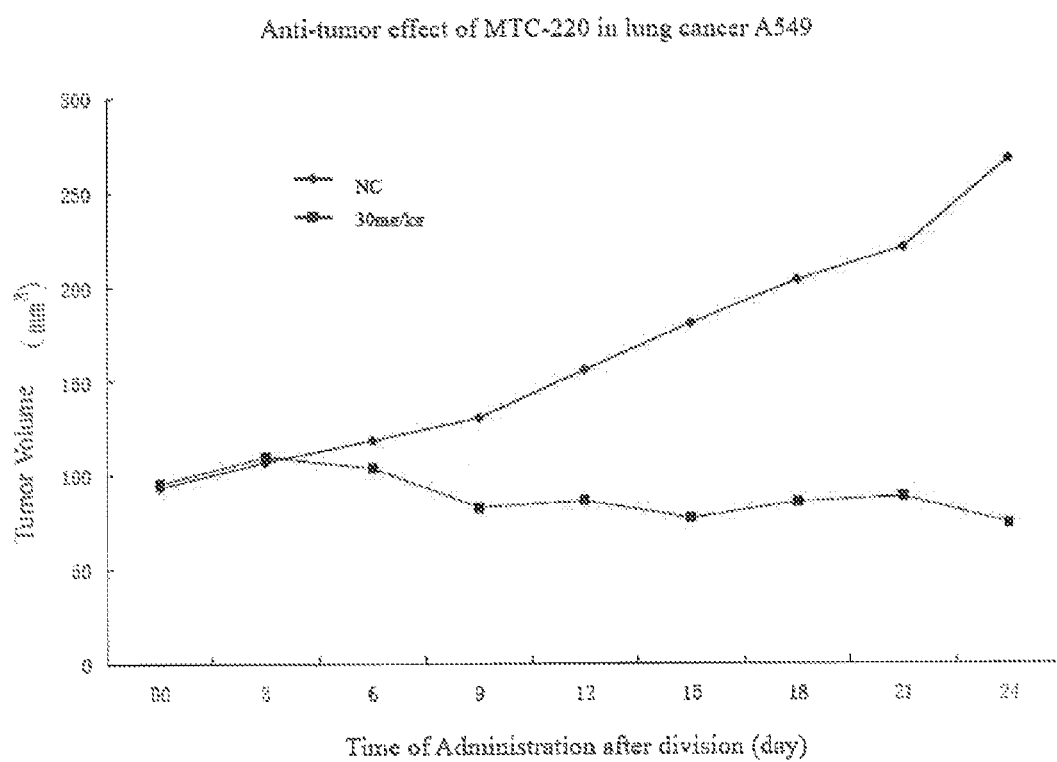
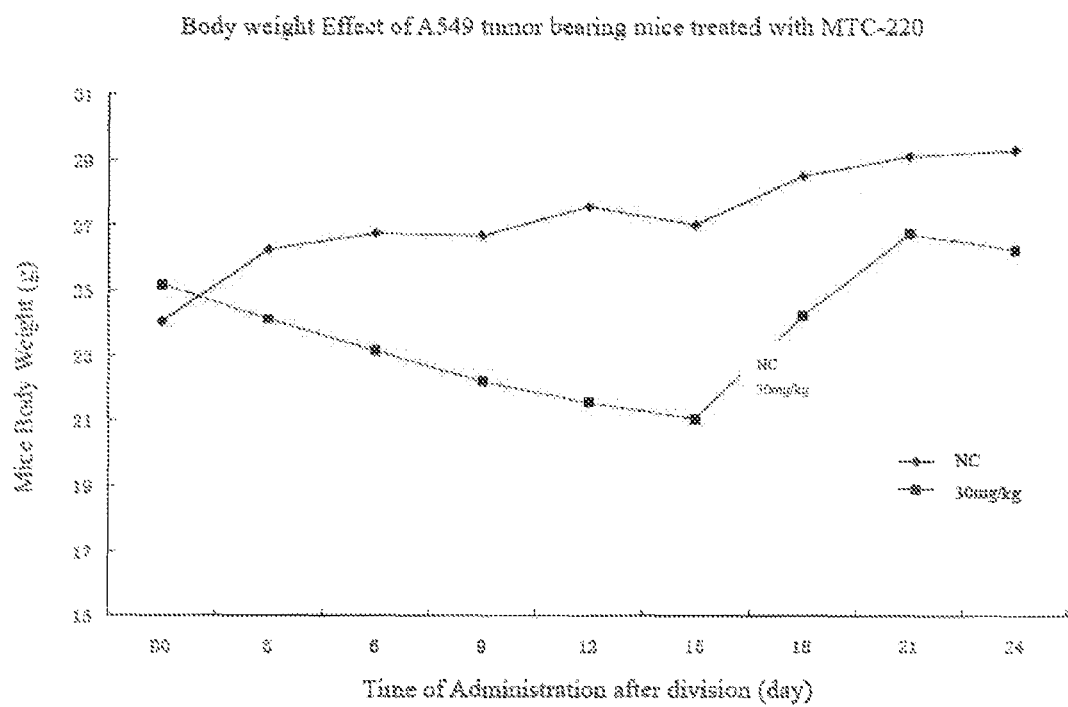


Figure 19

**Figure 20**

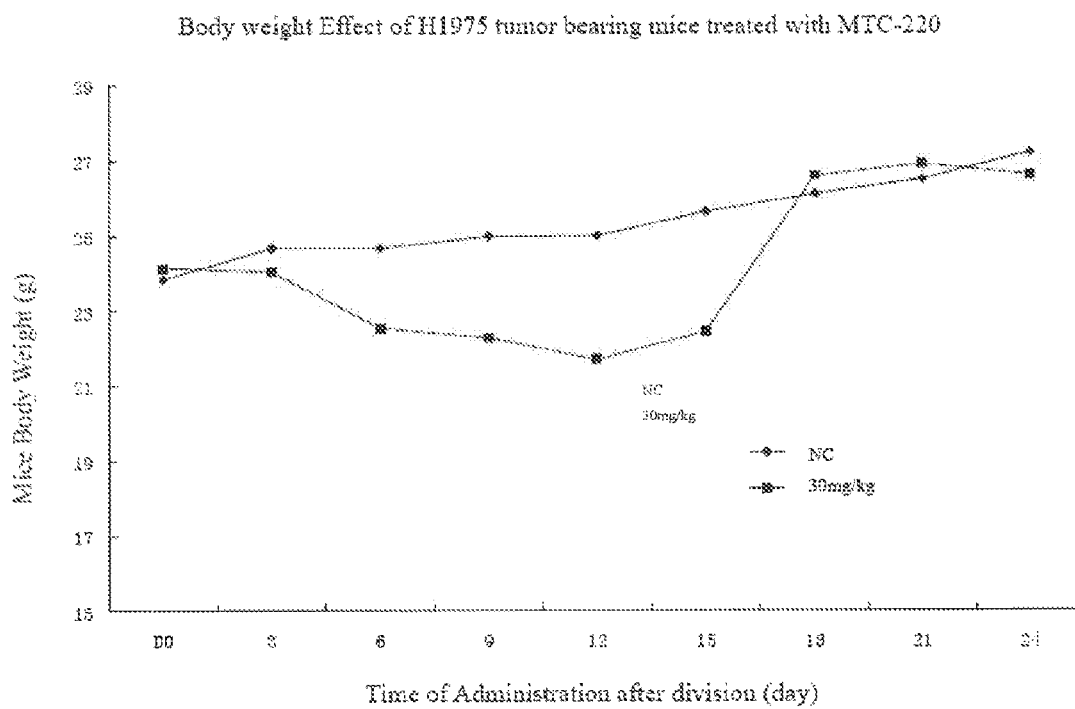


Figure 21

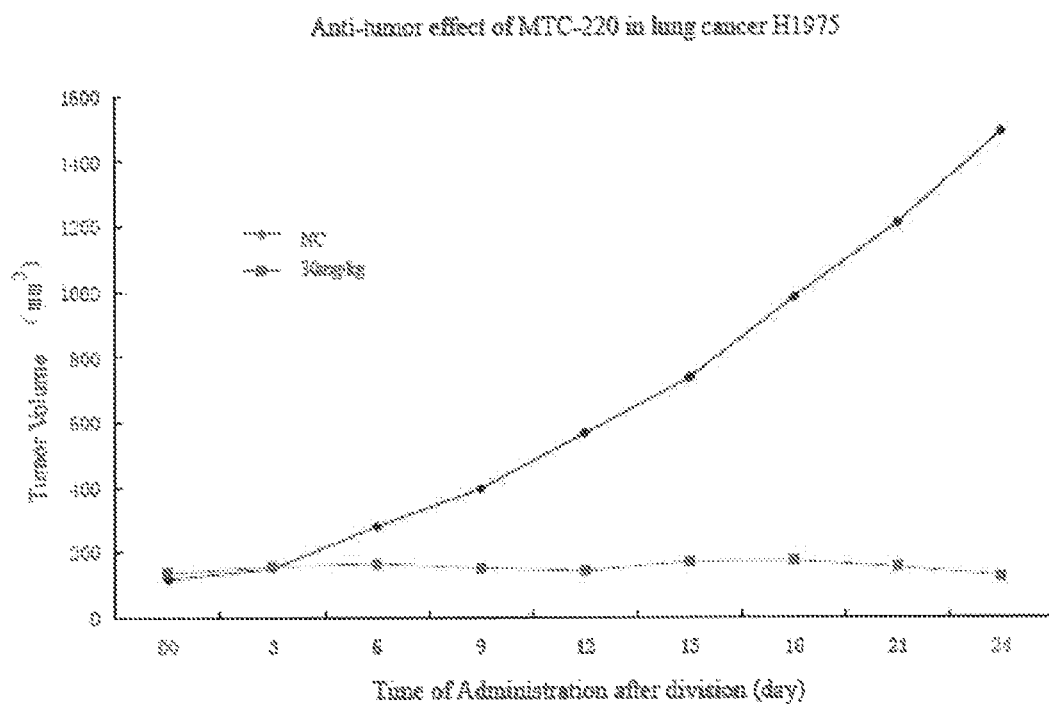


Figure 22

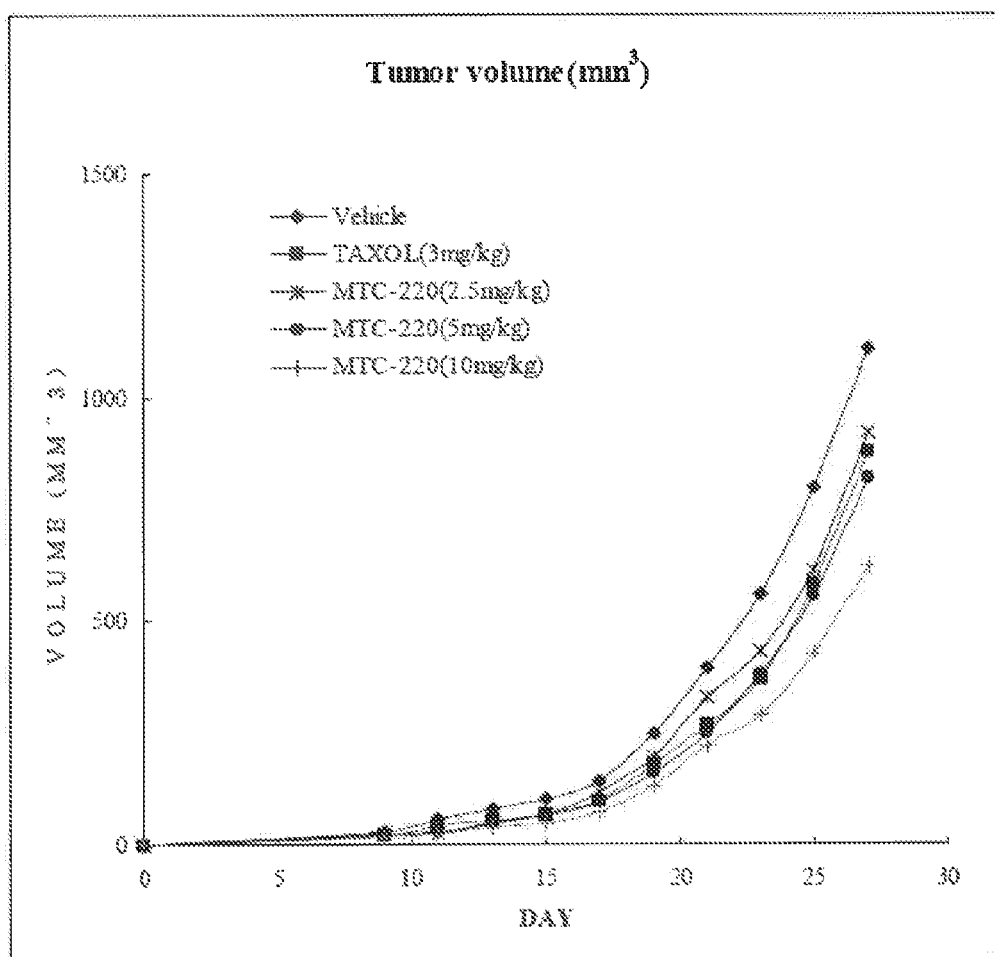
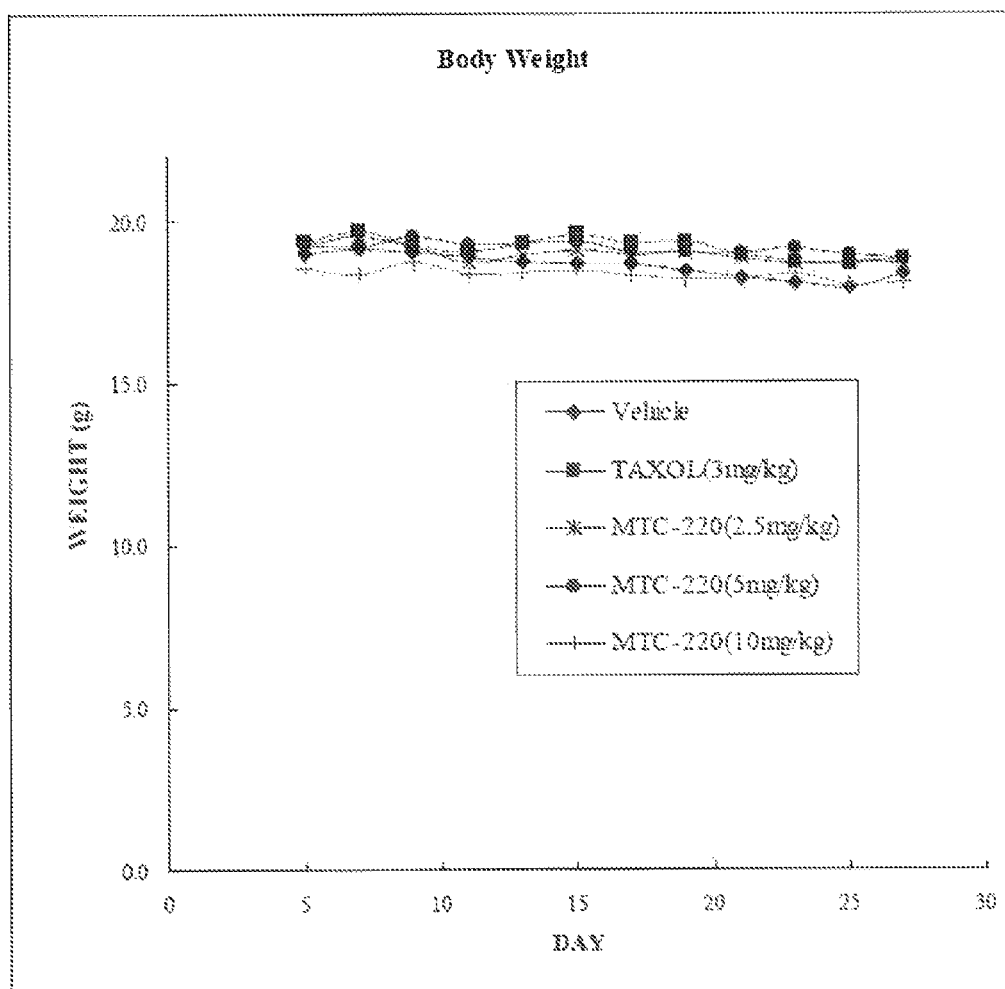


Figure 23

**Figure 24**

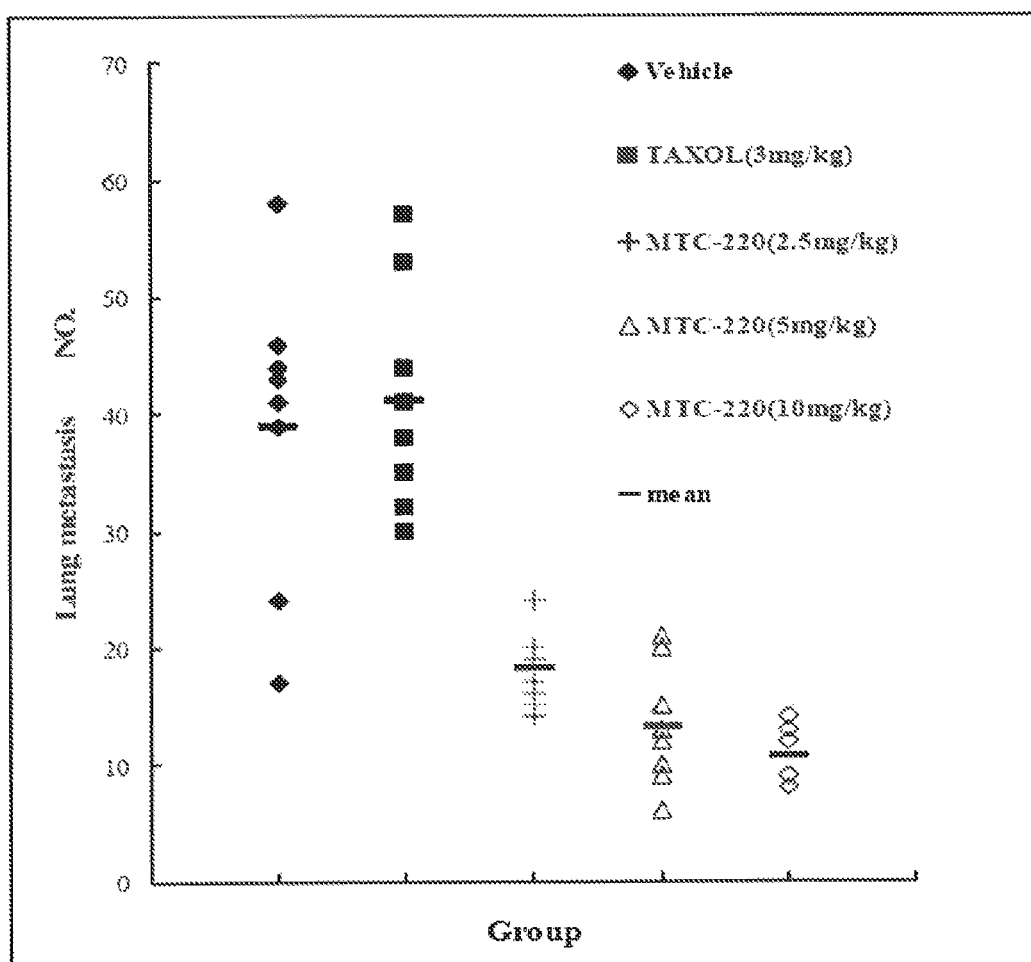


Figure 25

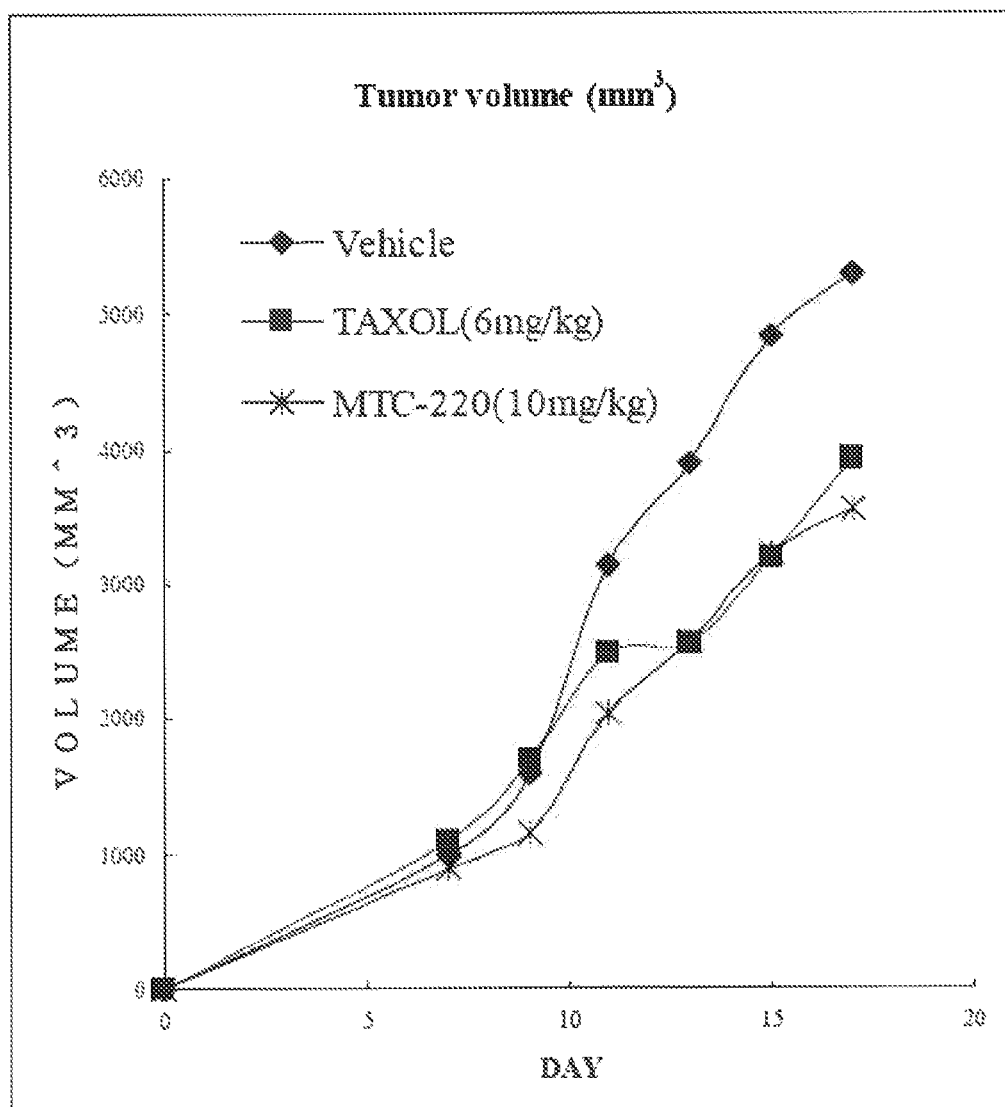


Figure 26

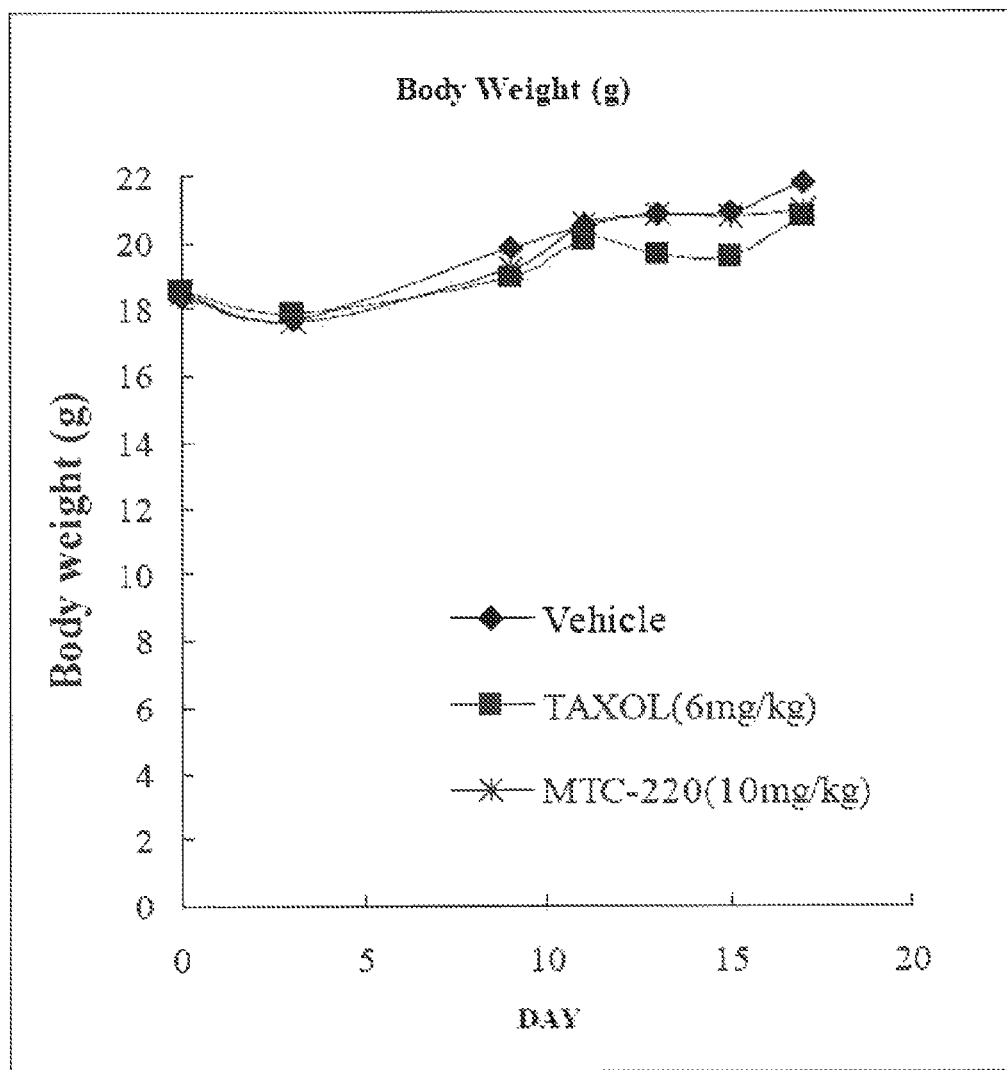


Figure 27

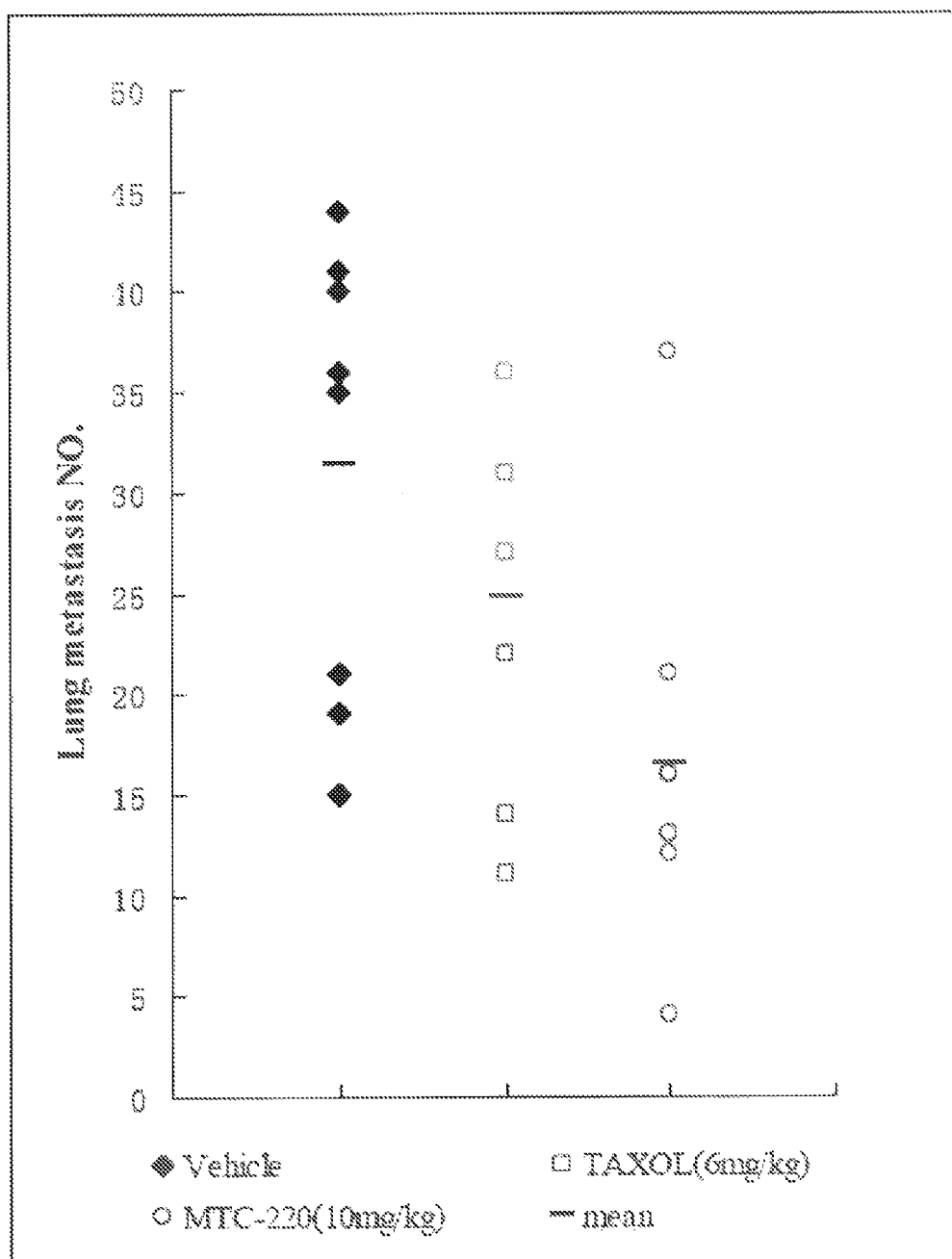


Figure 28

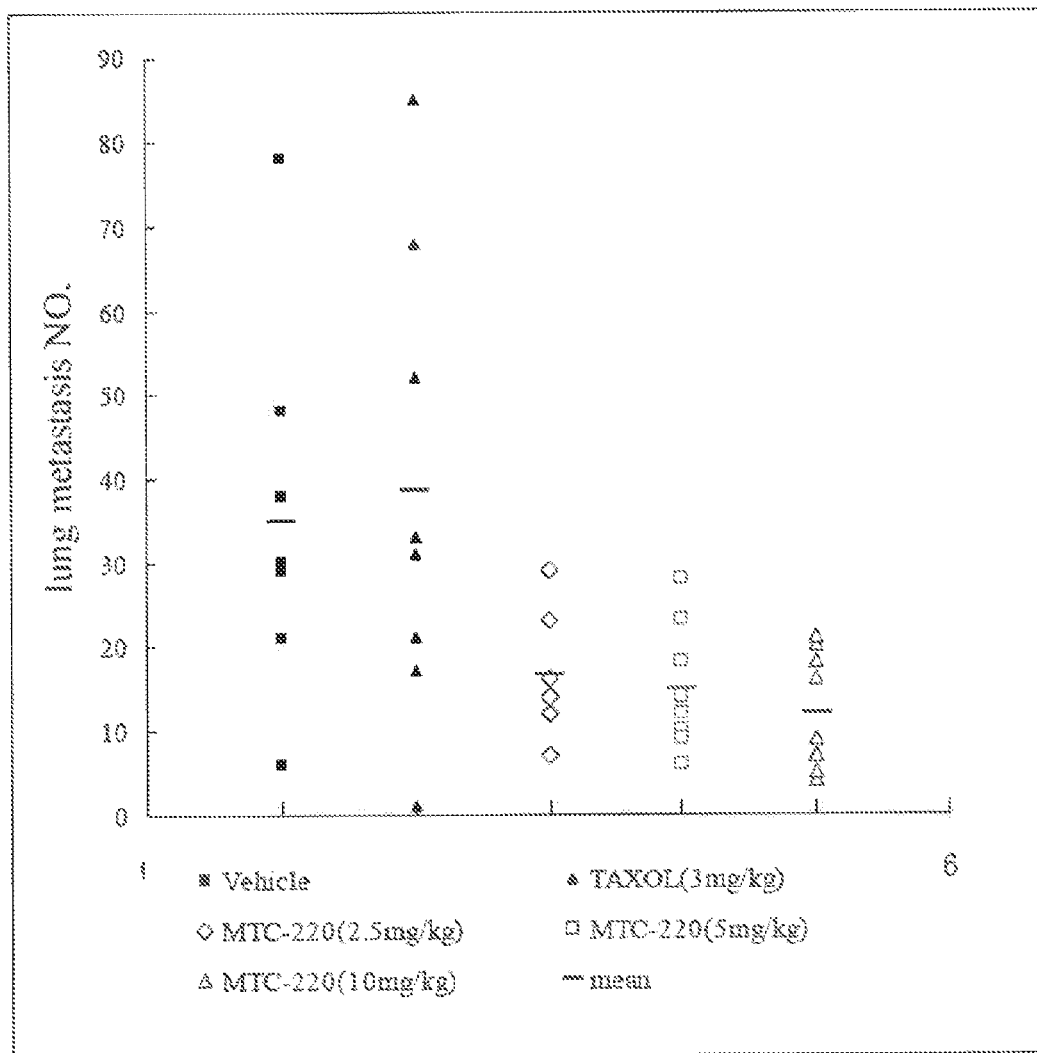


Figure 29

1

CHEMICAL SYNTHESIS AND ANTI-TUMOR AND ANTI-METASTATIC EFFECTS OF DUAL FUNCTIONAL CONJUGATE

FIELD

The present invention relates to a series of conjugates of paclitaxel and muramyl dipeptide derivatives, or docetaxel and muramyl dipeptide derivatives, and synthesis, use in cancer treatment thereof. The invention belongs to the field of medical technology.

BACKGROUND

Paclitaxel (also can be called TAXOL®), isolated from *Taxus brevifolia*^[1], was found to show anti-tumor activity by US National Cancer Institute (NCI). Premier mechanistic study indicated that paclitaxel is a mitotic inhibitor, which arrest the growth of cancer cells at G2 and M stage by promoting polymerization and depolymerization of cancer cell microtubule, then preventing formation of spindle in cancer cell^[2]. Further mechanistic study indicated paclitaxel can also be used as bacterium lipopolysaccharide (LPS) analogue, which exerts its anti-tumor effect by affecting or changing the function of macrophages in immune system, for example, by inducing the expression of tumor necrosis factor α (TNF- α) and interleukin-1 (IL-1) in macrophages^[3, 4]. Furthermore, it shows anti-tumor effect by activating MAP-2 kinase, and/or promoting tyrosine phosphorylation of cancer cells^[5, 6].

Muramyl dipeptide (N-acetylmuramyl-L-alanyl-D-isoglutamine, MDP) is the minimal structural unit shows immunoadjuvant activity among mycobacterium cell wall peptidoglycans^[7, 8]. MDP, injected at the same time with or before the injection of antigen, will enhance immune response or change immune response type. Furthermore, Muramyl

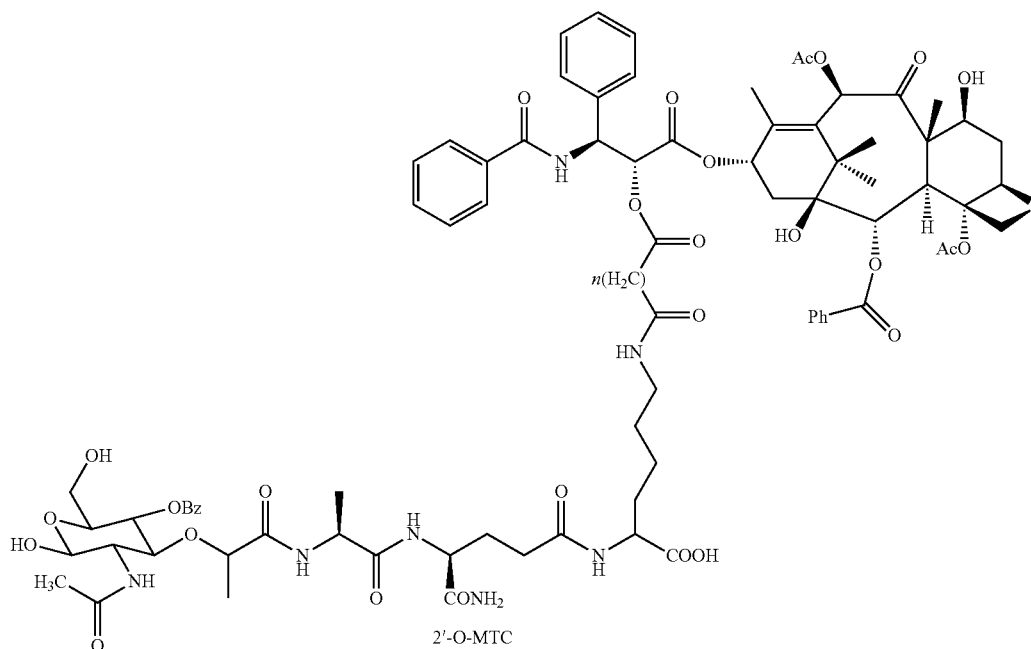
2

dipeptide shows other activities, such as nonspecific resistance to infection caused by, for example, *pneumobacillus*, *colibacillus*, *pseudomonas aeruginosa*, *mononucleosis listeria*, and/or *tritirachium album* etc, nonspecific resistance to, for example, fibrosarcoma and hepatoma etc, and immunoregulation^[9-13]. Studies also indicated that MDP together with lipopolysaccharide (LPS) can significantly stimulates the cytokines expression of macrophage^[14-16].

Based on these, we expected that paclitaxel together with muramyl dipeptide may show synergistic effect as well. We are the first to propose the new idea that bonding chemotherapy drug paclitaxel and immunostimulants muramyl dipeptide to form a series of conjugates. Biological tests are carried out to prove effectiveness of the new idea, which combines chemotherapy and immunotherapy to realize anti-tumor and anti-metastatic effects^[17].

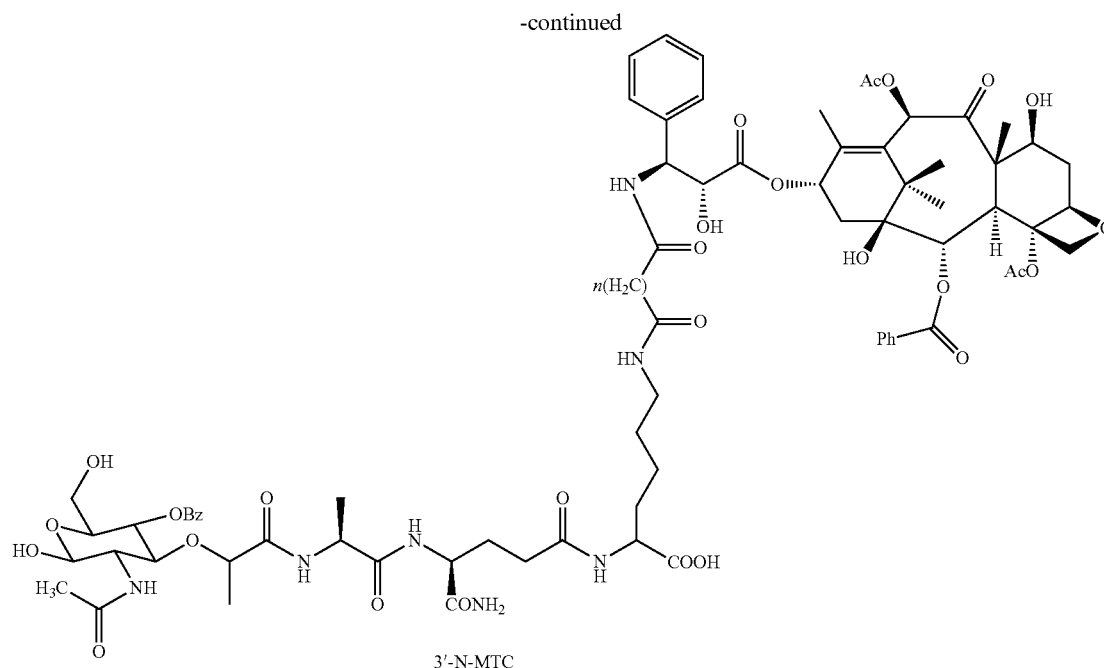
Applicants disclosed two types of conjugates in our previous patent application^[18], which were obtained by bonding muramyl dipeptide with paclitaxel 2'-hydroxy (2'-O-MTC, Structure 1), or with 3'-amino of 3'-N benzoyl paclitaxel (3'-N-MTC, Structure 1). In in vitro tests, Applicants found that 2'-O-MTC conjugate not only maintained anticancer activity of paclitaxel, but also assisted macrophages to produce α TNF- and IL-1 significantly, which means it potentially can inhibit metastasis. However, the activity of 3'-N-MTC conjugate was not significant. Based on that, we preliminarily determined the optimal position of conjugates for bonding would be paclitaxel's 2'-hydroxyl group. Unfortunately, 2'-O-MTC conjugate did not show desired results in vivo, which might depend on the physicochemical properties or the pharmaceutical properties of the molecule. To continue this design concept used in the new drug discovery, Applicants optimized the 2'-O-MTC conjugate by simplifying structures of muramyl dipeptide molecules, and obtained a new series of 2'-O-MTC analogues showing significant anti-tumor and anti-metastasis activities in vivo, which means they can be developed as antitumor drugs. Disclosed herein are the aforementioned new series of 2'-O-MTC analogues.

Structure I shows the two conjugates of muramyl dipeptide with paclitaxel disclosed in Applicants' previous patent application



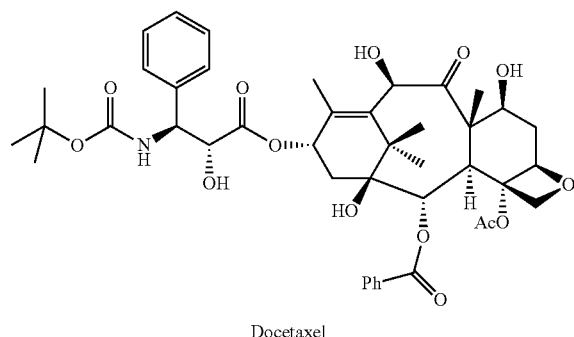
3

4



Paclitaxel is a taxanes antineoplastic drug, while docetaxel (Structure 2), a semisynthetic derivative of Paclitaxel, is another important member of taxanes antineoplastic drug which shows inhibitory activities against terminal breast cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, liver cancer, head and neck tumors. Current research indicated that docetaxel induces the apoptosis of cancer cell by promoting microtubule to form stable polymer, inhibiting depolymerization^[19], and furthermore inhibiting mitosis and proliferation of cancer cell^[20]. Research also discovered that docetaxel can make the tumor cell stop at G2/M stage by up regulating Bax protein expression and down regulating Bcl-2 protein expression^[21]. Based on this, the disclosure of this application involves replacing paclitaxel in the original conjugates with docetaxel to form conjugates of docetaxel-muramyl dipeptides (MDC), which also showed anti-tumor activities.

Structure 2



Muramyl dipeptide shows broad biological activities, and attracts great interest when discovered. However, muramyl dipeptide shows several side effects, such as immunogen induced allergic reactions, fever, inflammation and sleepi-

ness, which limit its clinical application. In order to find muramyl dipeptide analog with higher activity and fewer side effects, scientists have synthesized hundreds of muramyl dipeptide simplifiers or analogues, and studied their biological activities. L-threonine-Muramyl dipeptide is obtained by replacing L-alanine in muramyl dipeptide molecule with L-threonine, which shows higher immunoadjuvant activity than that of the muramyl dipeptide, but pyrogen is 100 times lower. When used as a vaccine adjuvant, L-threonine-Muramyl dipeptide doesn't stimulate macrophages and anti-inflammatory effects, but stimulates the immune response of the administered antigen, so it can be an ideal vaccine adjuvant because its activity and side effect can be effectively separated^[22].

Murabutide is obtained by introducing muramyl dipeptide to long lipotropic chain. Murabutide can enhance non-specific anti-bacterial and anti-viral infection of host immune system, and induce activity of colony stimulating factor. Also, it is well tolerated by human^[23-26]. Compared to other exogenous immunomodulators, Murabutide is non-pyrogenicity and promotes cytokines, both synergetically and selectively, to release Th1 cytokine, and Murabutide does not cause inflammatory response^[27, 28]. Furthermore, Murabutide combined with IFN- α or IL-2 can significantly enhance the anti-tumor activities of the cytokines, hence improve the anti-viral and anti-inflammatory effect of IFN- α ^[29, 30]. Murabutide can regulate function of macrophage^[31]. It can also be used in the treatment of chronic hepatitis C (HCV), because of the synergistic effect shown in vitro when combined with IFN- α ^[32].

Muramyl tripeptidephosphatidylethanolamine (MTP-PE) is obtained by introducing lipophilic long chain to muramyl dipeptides through phosphate bond. MTP-PE can activate monocytes and macrophages, then kill tumor cells. MTP-PE encapsulated in liposomes (L-MTP-PE), injected intravenously, is mainly directed to activate the macrophages in lung, liver and spleen^[33], wherein its activities is increased by ten to hundreds times, and pyrogenicity is significantly reduced. Two hours after being intravenously injected to

metastatic melanoma patients, tumor necrosis factor in plasma increased in sixteen times, and the level of neopterin and interleukin was effectively improved^[34].

MDP-Lys (L18) is obtained by introducing lipophilic long chain to muramyl dipeptides through lysine. MDP-Lys (L18) can enhance the production of cytokines such as CSFs, IL-1, IL-6, tumor necrosis factor (TNF- α) etc, which play important role in regulation of the hematopoietic system^[35, 36]. In addition, MDP-Lys (L8) has a strong anti-infection, anti-tumor activity^[37].

MDP-C is obtained by introducing aromatic conjugate system to muramyl dipeptides through lysine. MDP-C can induce macrophage to generate cytotoxic activity against P388 leukemia cells, it can also induce Tlymphocytes (CTLs) to generate cytotoxic activity against mastocytoma P815. It is reported that the MDP-C stimulates mouse bone marrow dendritic cells (BMDCs) to produce cytokines IL-2 and IL-12 (interleukin), and it also can be used as effective immunopotentiator for it shows activity on stimulating cytotoxic Tlymphocytes to produce interferon- γ . Low doses of MDP-C can significantly and synergistically promote proliferation of mouse spleen lymphocyte induced by Concanavalin A (ConA). In addition, MDP-C can increase the expression of bone marrow dendritic cell surface molecules, such as CD11c, MHC and cell adhesion molecule-1. Also, MDP-C, in vitro, can significantly enhance, through producing antibodies and specific hepatitis B virus surface antigen (HBsAg) Tcell response, the response of immune system to the HBsAg in hepatitis B virus transgenic mice^[38, 39].

Adamantantylamide dipeptide (AdDP) is obtained by bonding carboxyl teminal of dipeptide fragment in muramyl dipeptide molecule with amantadine. AdDP is safe, and shows anti-virus infection activity. Compared with other MDP analogues, its bioavailability is higher^[40]. AdDP can enhance the humoral immunity both in BALB/c mice and rabbit when administered with protein immunogen orally or peritoneally^[41].

Chemists also obtained muramyl dipeptide sugar-free ring analogs by synthesis or isolating from natural product, such as FK-156 and FK-565. They show anti-infection, anti-viral and anti-tumor activities^[42].

REFERENCE

- [1] Mansukhlal C. Wani, Harold Lawrence Taylor, Monroe E. Wall, Philip Coggon, Andrew T. McPhail; Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*; J. Am. Chem. Soc.; 1971, 93(9), 2325-2327.
- [2] Peter B. Schiff and Susan B. Horwitz; Taxol stabilizes microtubules in mouse fibroblast cells; Proc. Natl. Acad. Sci. USA; 1980, 77(3), 1561-1565.
- [3] A. H. Ding, F. Porteu, E. Sanchez, and C. F. Nathan; Shared actions of endotoxin and taxol on TNF receptors and TNF release; Science; 1990, 20, 370-372.
- [4] Christian Bogdan and Aihao Ding; Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor α and interleukin-1 in macrophages; Journal of Leukocyte Biology; 1992, 52, 119-121.
- [5] B. Brugg and A. Matus; Phosphorylation determines the binding of microtubule-associated protein 2 (MAP2) to microtubules in living cells; J. Cell Biol.; 1991, 114 (4), 735-743.
- [6] Carboni, J., Singh, C., Tepper, M.; Cancer Institute Workshop on Taxol and Taxus, Alenandria, V. A.; NCI, 1992.

- [7] Ellouz F., Adam A., Ciorbaru R., et al; Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives; Biochem. Biophys. Res. Commun.; 1974, 59, 1317-1325.
- [8] Adam, A., Ciorbaru, R., Ellouz, F., Petit, J. F. and Lederer, E.; Adjuvant activity of monomeric bacterial cell wall peptidoglycans; Biochem. Biophys. Res. Commun.; 1974, 56(3), 561-567.
- [9] F. Audibert, L. Chédid, P. Lefrancier, J. Choay; Distinctive adjuvanticity of synthetic analogs of mycobacterial water-soluble components; Cellular Immunology; 1976, 21, 243-249.
- [10] M. A. Parant, F. M. Audibert, L. A. Chedid, M. R. Level, P. L. Lefrancier, J. P. Choay, and E. Lederer; Immunostimulant activities of a lipophilic muramyl dipeptide derivative and of desmuramyl peptidolipid analogs; Infect. Immun.; 1980, 27, 826-831.
- [11] Adam A., Petit J. F., Chedid L.; Influence of a synthetic adjuvant (MDP) on qualitative and quantitative changes of serum globulins; Immunology; 1978, 35(6), 963-970.
- [12] Dietrich F. M., Hochkeppel H. K., Lukas B.; Enhancement of host resistance against virus infections by MTP-PE, a synthetic lipophilic muramyl peptide—increased survival in mice and guinea pigs after single drug administration prior to infection, and the effect of MTP-PE on interferon levels in sera and lungs; Int. J. Immunopharmacol.; 1986, 8, 931-932.
- [13] Adam A., Lederer E.; Muramyl peptides: immunomodulators, sleep factors, and vitamins; Med. Res. Rev., 1984, 4(2), 111-152.
- [14] Anton V. Gorbachev, Nancy A. Dilulio, and Robert L.; Fairchild IL-12 augments CD81 T cell development for contact hypersensitivity responses and circumvents Anti-CD154 antibody-mediated inhibition; The Journal of Immunology, 2001, 167, 156-162.
- [15] Alexandre A. Vetcher, Marek Napierala, Ravi R. Iyer, Paul D. Chastain, Jack D. Griffith, and Robert D.; Wells sticky DNA, a long GAA-GAA-TTC triplex that is formed intramolecularly, in the sequence of intron 1 of the frataxin gene; J. Biol. Chem.; 2002, 277, 39217-39227.
- [16] C. L. Contel, N. Temime, D. J. Charron, and M. A. Parant; Modulation of lipopolysaccharide-induced cytokine gene expression in mouse bone marrow-derived macrophages by muramyl dipeptide; The Journal of Immunology; 1993, 150, 4541-4549.
- [17] Xuqin Li, Junli Yu, Song Xu, Nan Wang, Hongzhen Yang, Zheng Yan, Guifang Cheng, Gang Liu; Chemical conjugation of muramyl dipeptide and paclitaxel to explore the combination of immunotherapy and chemotherapy for cancer, Glycoconj J.; 2008, 25(5), 415-425.
- [18] Patent No. 200510081265X.
- [19] Toshiyuki Harada, Shigeaki Ogura, Koichi Yamazaki, Ichiro Kinoshita, Tomoo Itoh, Hiroshi Isobe, Katsushige Yamashiro, Hirotohi Dosaka-Akita, Masaharu Nishimura; Predictive value of expression of P53, Bcl-2 and lung resistance-related protein for response to chemotherapy in non-small cell lung cancers; Cancer Science; 2005, 94(4), 394-399.
- [20] David L. Morse, Heather Gray, Claire M. Payne, and Robert J. Gillies; Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells; Mol Cancer Ther, 2005, 4, 1495-1504.
- [21] Yu Q, Gao J. X., He X. S., et al; Docetaxel induces apoptosis and regulates expressions of bax and bcl-2 protein in human breast carcinoma MCF-7 Cells; Cancer Res. Pre. Treatment, 2006, 33(6), 388-390.

- [22] Deborah A. Epstein, Noeline E. Byars, Anthony C. Allison; New adjuvants for vaccines containing purified protein antigens; *Advanced Drug Delivery Reviews* 1990, 4, 233-253.
- [23] L. A. Chedid, M. A. Parant, F. M. Audibert, G. J. Riveau, F. J. Parant, E. Lederer, J. P. Choay, and P. L. Lefrancier, Biological activity of a new synthetic muramyl peptide adjuvant devoid of pyrogenicity; *Infection and Immunity*; 1982, 35, 417-424.
- [24] Chomel J. J., Simon-Lavoine N., Thouvenot D., Valette M., Choay J., Chedid L., Aymard M.; Prophylactic and therapeutic effects of murabutide in OF1 mice infected with influenza A and B viruses; *International Journal of Immunopharmacology*; 1985, 7(3), 346-347.
- [25] George M. Bahr, Edith Darcissac, Dorian Bevec, Peter Dukor, Louis Chedid; Immunopharmacological activities and clinical development of muramyl peptides with particular emphasis on murabutide; *International Journal of Immunopharmacology*; 1995 17(2), 117-131.
- [26] A. Galelli, P. Lefrancier, and L. Chedid; Colony-stimulating activity induced by synthetic muramyl peptides: variation with chemical structure and association with anti-infectious activity; *Infection and Immunity*; 1984, 46, 495-500.
- [27] George M. Bahr, Edith Darcissac, Philippe R. Pouillart, Louis A. Chedid; Synergistic effects between recombinant interleukin-2 and the synthetic immunomodulator murabutide: selective enhancement of cytokine release and potentiation of antitumor activity; *Journal of Interferon and Cytokine Research*; 1996, 16(2), 169-178.
- [28] Edith C. A. Darcissac, George M. Bahr, Philippe R. Pouillart, Gilles J. Riveau, Monique A. Parant; Selective potentiation of cytokine expression in human whole blood by murabutide, a muramyl dipeptide analogue; *Cytokine*, 1996, 8, 658-666.
- [29] George M. Bahr, Philippe R. Pouillart, Louis A. Chedid; Enhancement in vivo of the anti-inflammatory and antitumor activities of type I interferon by association with the synthetic immunomodulator murabutide; *Journal of Interferon and Cytokine Research*; 1996, 16(4), 297-306.
- [30] Philippe R. Pouillart, Francoise M. Audibert, Louis A. Chedid, Pierre L. Lefrancier, George M. Bahr; Enhancement by muramyl peptides of the protective response of interferon- α/β against encephalomyocarditis virus infection; *International Journal of Immunopharmacology*; 1996, 18(3), 183-192.
- [31] Gilles J. Riveau, Beatrice G. Brunel-Riveau, Francoise M. Audibert, Louis A. Chedid; Influence of a muramyl dipeptide on human blood leukocyte functions and their membrane antigens; *Cellular Immunology*; 1991, 134, 147-156.
- [32] E. C. A. Darcissac, V. Vidal, M. Guillaume, J. J. Thebault, G. M. Bahr; Clinical tolerance and profile of cytokine induction in healthy volunteers following the simultaneous administration of IFN- α and the synthetic immunomodulator murabutide; *Journal of Interferon and Cytokine Research*; 2001, 21(9), 655-661.
- [33] (a) Nardin A., Lefebvre M. L., Labroquere K., Faure O., Abastado J. P.; Liposomal muramyl tripeptide phosphatidylethanolamine: tTargeting and activating macrophages

- for adjuvant treatment of osteosarcoma; *Current Cancer Drug Targets*; 2006, 6, 123-133.
- (b) Meyers Paul A., Schwartz Cindy L., et al; A randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate; *J. Clin. Oncol.*; 2005, 23(9), 2004-2011.
- [34] Liebes L., Walsh C. M., Chachoua A., et al; Modulation of monocyte functions by muramyl triptide phosphatidylethanolamine in a phase II study in patients with metastatic melanoma; *J. Natl. Cancer. Inst.*; 1992, 84, 694-699.
- [35] Y. Osada, T. Otani, M. Sato, T. Une, K. Matsumoto, and H. Ogawa; Polymorphonuclear leukocyte activation by a synthetic muramyl dipeptide analog; *Infection and Immunity*; 1982, 38, 848-854.
- [36] Kenji Namba, Eiko Yamamura, Hironobu Nitani, Tsuyoshi Otani, Ichiro Azuma; Romurtide, a synthetic muramyl dipeptide derivative, promotes megakaryocytopoiesis through stimulation of cytokine production in nonhuman primates with myelosuppression; *Vaccine*, 1997, 15(4), 405-413.
- [37] Ichiro Azuma, Tsukasa Seya; Development of immunoadjuvants for immunotherapy of cancer; *International Immunopharmacology*; 2001, 1(7), 1229-1392.
- [38] Hong-Zhen Yang, Song Xu, Xue-Yan Liao, Suo-De Zhang, Zheng-Lun Liang, Bai-He Liu, Jin-Ye Bai, Chao Jiang, Jian Ding, Gui-Fang Cheng, and Gang Liu; A novel immunostimulator, N₂-[α -O-Benzyl-N-(acetylmuramyl)-1-alanyl-d-isoglutaminyl]-N₆-trans-(m-nitrocinnamoyl)-1-lysine, and its adjuvancy on the hepatitis B surface antigen; *J. Med. Chem.*; 2005, 48(16), 5112-5122.
- [39] Patent No. CN1609118A.
- [40] P. Walder, E. Buchar, Z. Machková, T. Vrba, M. Flegel, I. Janků, K. Mas'ek; Pharmacokinetic profile of the immunomodulating compound adamantylamide dipeptide (AdDP), a muramyl dipeptide derivative in mice; *Immunopharmacology and Immunotoxicology*, 1991, 13 (1 and 2), 101-119.
- [41] Pablo D. Becker, Ricardo S. Corral, Carlos A. Guzmán, Saul Grinstein; Adamantylamide dipeptide as effective immunoadjuvant in rabbits and mice; *Vaccine*; 2001, 19(32), 4579-4903.
- [42] A. M. Kolodziejczyk, A. S. Kolodziejczyk, S. Stoev; New convenient synthesis of immunostimulating peptides containing meso-diaminopimelic acid Syntheses of FK-565 and FK-156; *International Journal of Peptide and Protein Research*; 1992, 39(4), 382-387.

DETAILED DESCRIPTION OF THE INVENTION

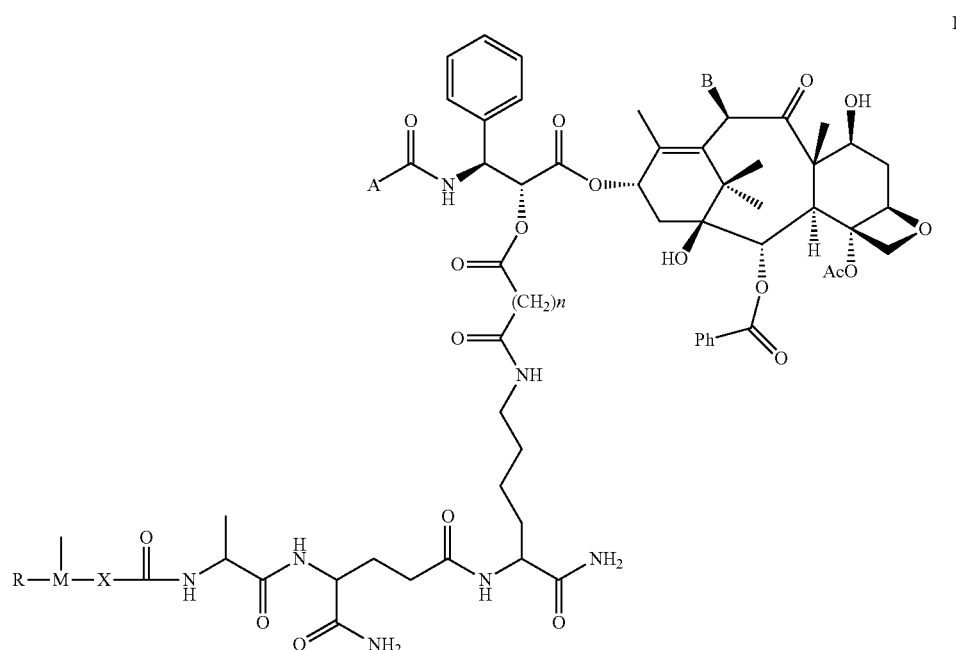
The technical problem to be solved by the present application is to provide a compound having anti-tumor and anti-metastasis synergy activities.

The second technical problem to be solved by the present application is to provide a method for the preparation of the compound.

The third technical problem to be solved by the present application is to provide pharmaceutical composition comprising the compounds.

A further technical problem to be solved in the present application is to apply the compound in the preparation of anti-tumor and anti-metastasis synergy drugs.

Provided is a compound of formula I, and/or a pharmaceutically acceptable salt thereof,



wherein, when A is phenyl, B is acetoxy; when A is tert-butoxy, B is hydroxy;

wherein, $n=2, 3, 4, 5, 6, 7, 8, 9, 10, 11$ or 12 .

In a preferred embodiment, $n=2, 3, 4, 5, 6, 7, 8, 9$ or 10 .

In another preferred embodiment, $n=2, 3, 4, 5, 6, 7$ or 8 .

In a further preferred embodiment, $n=2, 3, 4$ or 5 .

Wherein X is chosen from C_{1-6} alkyl, C_{1-6} alkylene and C_{1-6} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is independently chosen from oxygen, sulfur and nitrogen; or X is a single bond, which means M is connected to carbonyl directly.

In a preferred embodiment, X is chosen from C_{1-4} alkyl, C_{1-4} alkylene and C_{1-4} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is independently chosen from oxygen and sulfur; or X is a single bond, which means M is connected to carbonyl directly.

In another preferred embodiment, X is chosen from C_{1-3} alkyl, C_{1-3} alkylene and C_{1-3} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is oxygen; or X is a single bond, which means M is connected to carbonyl directly.

In a further preferred embodiment, X is chosen from $-C=C-$, $-CH_2-CH_2-$, $-O-CH_2-$ and single bond.

M can be substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, for example, M can be aryl or heteroaryl, the term "aryl" as disclosed herein refers to five to fourteen membered aromatic ring.

In one embodiment, M is chosen from five-membered aryl, six-membered aryl, nine-membered fused ring aryl, ten-membered fused ring aryl, thirteen-membered fused ring aryl and fourteen-membered fused ring aryl.

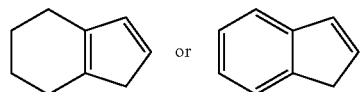
The term "five-membered aryl" as disclosed herein refers to



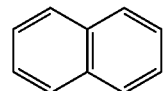
The term "six-membered aryl" as disclosed herein refers to



The term "nine-membered fused ring aryl" as disclosed herein refers to



The term "ten-membered fused ring aryl" as disclosed herein refers to



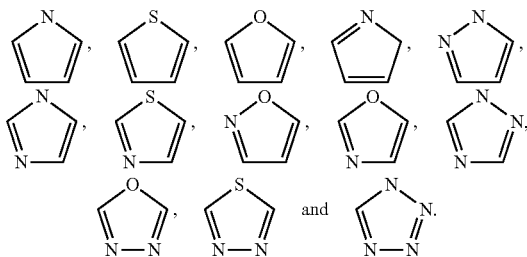
The term "heteroaryl" can be, for example, a heterocyclic aromatic ring comprising at least one, such as one, two, three, and four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur.

For another example, the "heteroaryl" can be five to fourteen membered heterocyclic aromatic ring comprising at least one, such as one, two, three, and four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur.

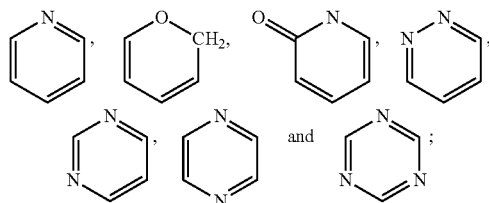
11

For a further example, the “heteroaryl” can be chosen from five-membered heterocyclic aromatic ring, six-membered heterocyclic aromatic ring, eight-membered fused heterocyclic aromatic ring, nine-membered fused heterocyclic aromatic ring, ten-membered fused heterocyclic aromatic ring, all of the aromatic ring mentioned above comprising at least one, such as one, two, three, and four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur.

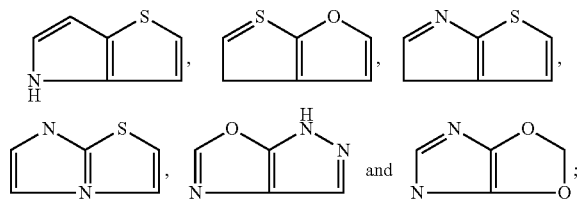
The term “five-membered heterocyclic aromatic ring” comprising at least one, for example one, two, three, or four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur, the five-membered heterocyclic aromatic ring disclosed herein is chosen from



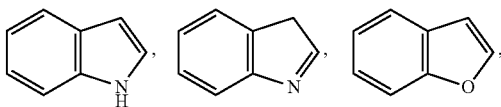
The term “six-membered heterocyclic aromatic ring” comprising at least one, for example one, two, three, or four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur, the six-membered heterocyclic aromatic ring disclosed herein is chosen from



The term “eight-membered fused heterocyclic aromatic ring” comprising at least one, for example one, two, three, or four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur, the eight-membered fused heterocyclic aromatic ring disclosed herein is chosen from

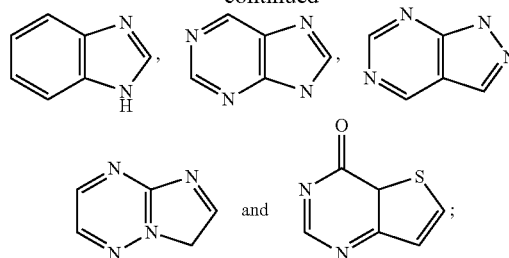


The term “nine-membered fused heterocyclic aromatic ring” comprising at least one, for example one, two, three, or four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur, the nine-membered fused heterocyclic aromatic ring disclosed herein is chosen from

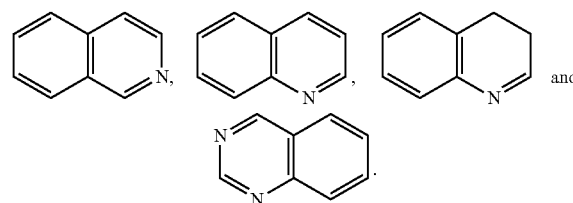


12

-continued



The term “ten-membered fused heterocyclic aromatic ring” comprising at least one, for example one, two, three, or four heteroatoms in the ring, wherein the at least one heteroatom is independently chosen from nitrogen, oxygen and sulfur, the ten-membered fused heterocyclic aromatic ring disclosed herein is chosen from



R refers to one or more groups, and R can be connected to M at any applicable point of attachment.

In one embodiment, R is chosen from hydrogen, substituted or unsubstituted straight or branched C_{1-6} alkyl, hydroxy, substituted or unsubstituted straight or branched C_{1-6} alkoxy, thiol, substituted or unsubstituted straight or branched C_{1-6} alkylthio, C_{1-6} alkoxy- C_{1-6} alkyl, amino, substituted or unsubstituted straight or branched C_{1-6} alkylamino includes mono-alkylamino or di-alkylamino, aldehyde group, substituted or unsubstituted straight or branched C_{1-6} alkylcarbonyl, carboxyl, substituted or unsubstituted straight or branched C_{1-6} alkylcarboxyl, carbamoyl, substituted or unsubstituted straight or branched C_{1-6} alkylamide, C_{2-6} alkene, halogen, nitro and cyano;

The substituent(s) on substituted C_{1-6} straight chain or branched chain described herein is independently chosen from hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro and cyano;

In one embodiment, R is chosen from hydrogen, substituted or unsubstituted straight or branched C_{1-4} alkyl, hydroxy, substituted or unsubstituted straight or branched C_{1-4} alkoxy, C_{1-4} alkoxy- C_{1-4} alkyl, thiol, substituted or unsubstituted straight or branched C_{1-4} alkylthio, amino, substituted or unsubstituted straight or branched C_{1-4} alkylamino includes mono-alkylamino or di-alkylamino, aldehyde group, substituted or unsubstituted straight or branched C_{1-4} alkylcarbonyl, carboxyl, substituted or unsubstituted straight or branched C_{1-4} alkylcarboxyl, carbamoyl, substituted or unsubstituted straight or branched C_{1-4} alkylamide, C_{2-4} alkene, halogen, nitro and cyano;

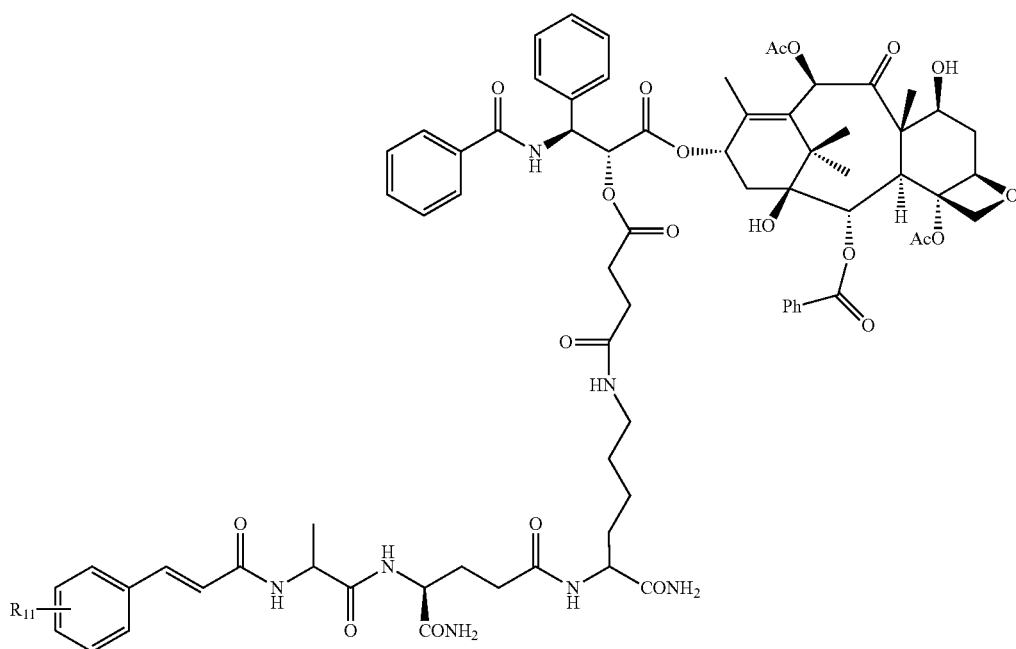
The substituent(s) on substituted straight or branched C_{1-4} chain described herein is chosen from hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, fluorine, chlorine, bromine, nitro and cyano;

In one embodiment, R is chosen from hydrogen, straight or branched C_{1-4} alkyl, hydroxy, straight or branched C_{1-4} alkoxy, thiol, straight or branched C_{1-4} alkylthio, amino, straight or branched C_{1-4} alkylamino, halogen, nitro and cyano;

In one embodiment, R is chosen from hydrogen, hydroxyl, thiol, amino, fluorine, chlorine, bromine, nitro, cyano, methyl, ethyl, n-propyl, iso-propyl, methoxy, ethoxy, n-propoxy and iso-propoxy;

13

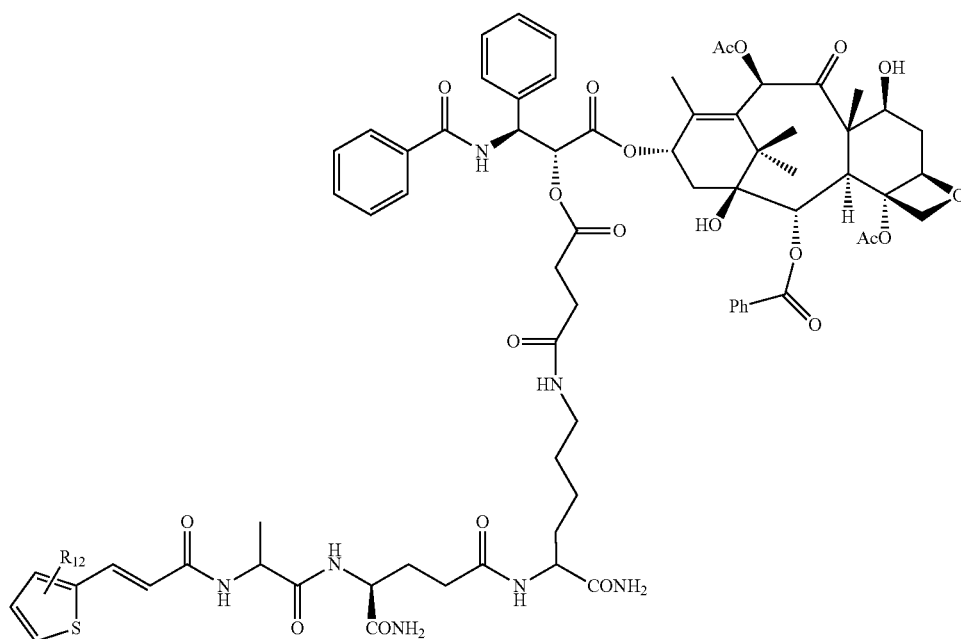
In one embodiment, the compound of formula I as disclosed herein is chosen from the compounds of formula IA as below:



IA

R_{11} refers to one or more groups, and R_{11} can be connected to phenyl at any applicable point of attachment. In one embodiment, R_{11} is independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the compound of formula I as disclosed herein is chosen from compounds of formula IB as below:



IB

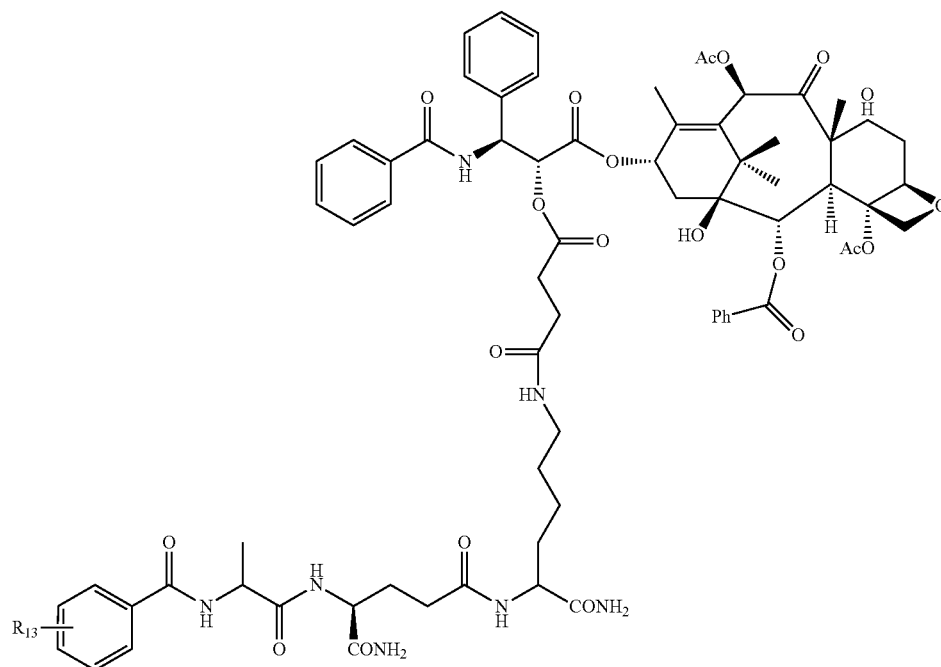
R_{12} refers to one or more groups, and R_{12} can be connected to thienyl at any applicable point of attachment. In one embodiment, R_{12} is independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the compound of formula I as disclosed herein is chosen from compounds of formula IC as below:

15

16

IC



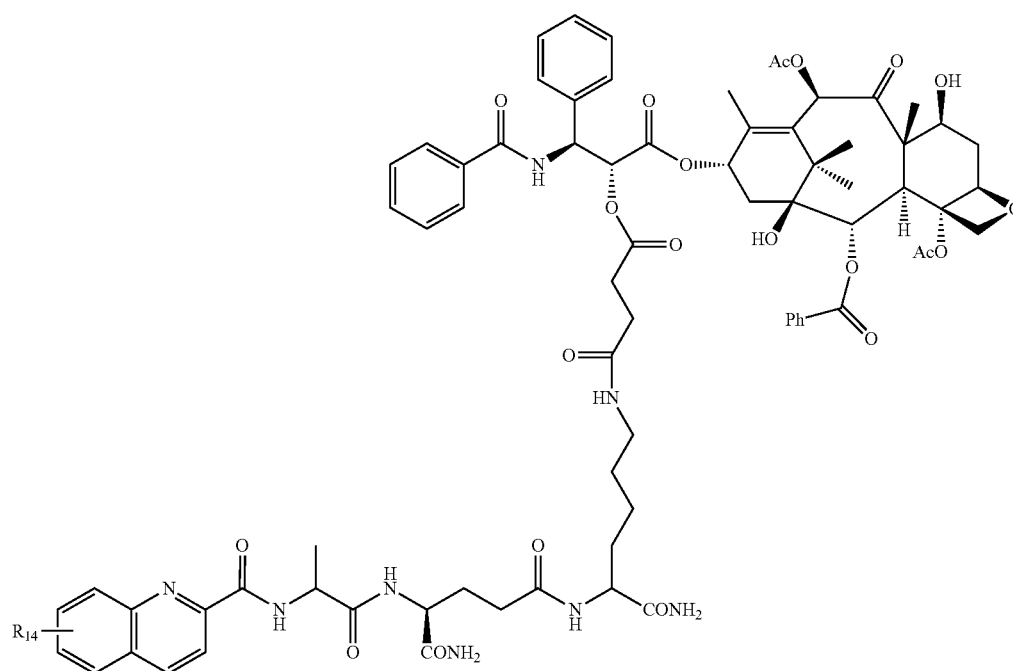
R_{13} refers to one or more groups, and R_{13} can be connected to phenyl at any applicable point of attachment. In one embodiment, R_{13} is independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the compound of formula I as disclosed herein is chosen from compounds of formula ID as below:

R_{14} refers to one or more groups, and R_{14} can be connected to quinolyl at any applicable point of attachment. In one embodiment, R_{14} is independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the compound of formula I as disclosed herein is chosen from compounds of formula IE as below:

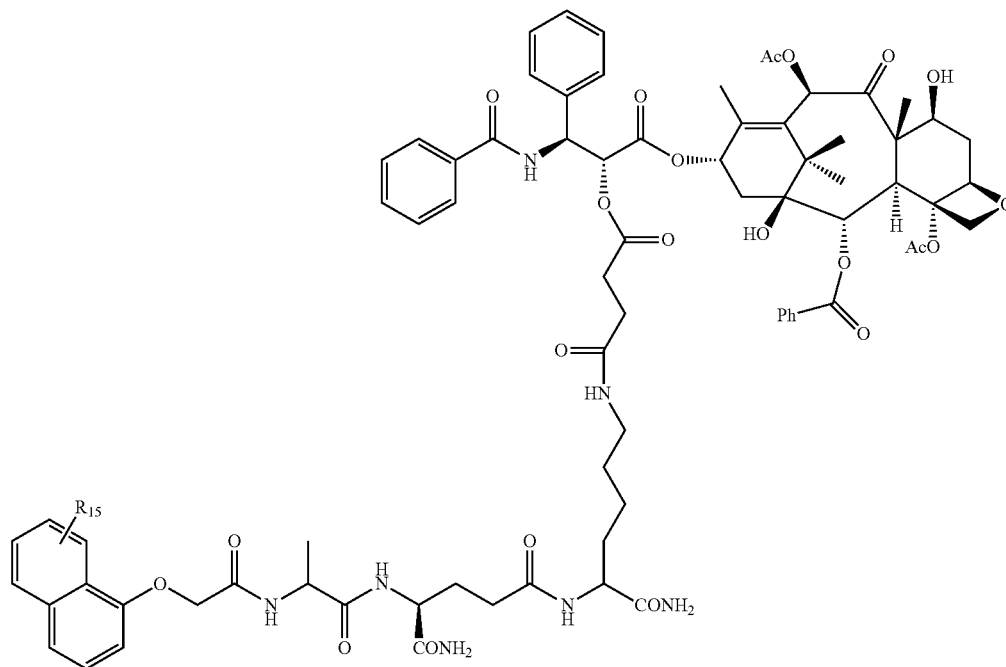
ID



17

18

IE



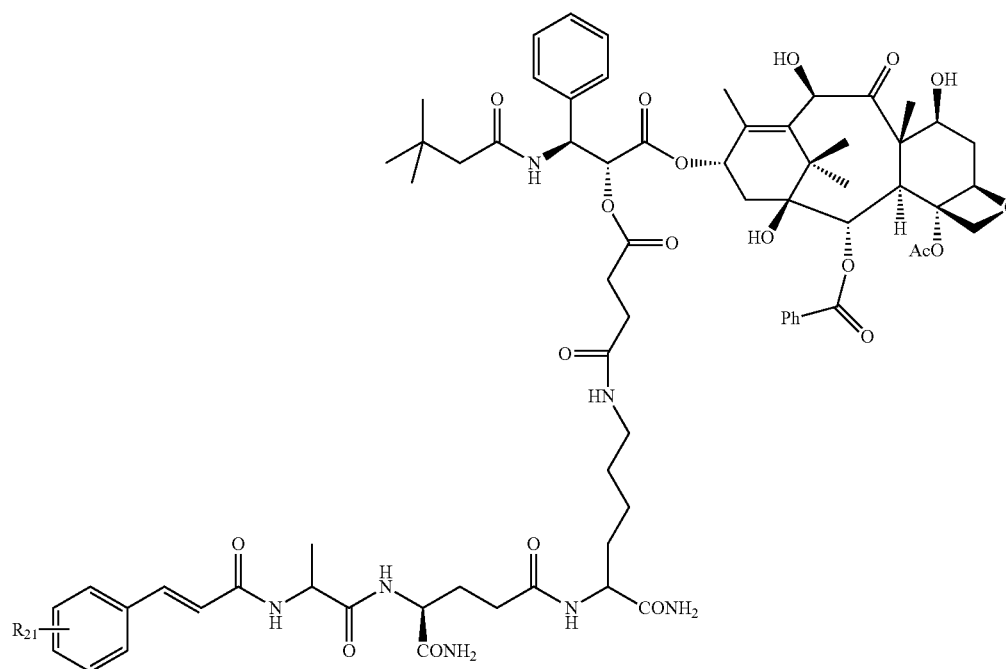
R_{15} is one or more groups, and R_{15} can be connected to naphthyl at any applicable point of attachment. In one embodiment, R_{15} is chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the compound of formula I as disclosed herein is chosen from compounds of formula IF as below:

R_{21} refers to one or more groups, and R_{21} can be connected to phenyl at any applicable point of attachment. In one embodiment, R_{21} is chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} alkoxy- C_{1-4} alkyl.

In one embodiment, the straight or branched C_{1-6} alkyl described herein refers to the straight or branched C_{1-4} alkyl, or the straight or branched C_{2-5} alkyl. In another embodiment,

IF



19

the straight or branched C_{1-6} alkyl is chosen from methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, pentyl, neo-pentyl, iso-pentyl and hexyl. The straight or branched C_{1-4} alkyl described herein is preferably chosen from methyl, ethyl, n-propyl, iso-propyl, n-butyl, and tert-butyl. The straight or branched C_{2-5} alkyl described herein is preferably chosen from ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, pentyl, and iso-pentyl.

The substituent(s) on substituted straight or branched C_{1-6} alkyl described herein can be chosen from hydroxyl, sulfydryl, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro and cyano.

The substituent(s) on substituted straight or branched C_1-C_4 alkyl described herein can be chosen from hydroxyl, sulfydryl, amino, aldehyde group, carboxyl, carbamoyl, fluorine, chlorine, bromine, nitro and cyano.

The term " C_{2-6} alkene" as disclosed herein refers to alkene having two, three, four, five or six carbon atoms. It can be straight chain or branched chain. For example, C_{2-6} alkene can be chosen from vinyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 1-pentenyl and 1-hexenyl, C_{2-6} alkene is preferably chosen from C_{2-4} alkene.

The term "alkoxy" as disclosed herein refers to $-O$ -alkyl.

The term "halogen" as disclosed herein refers to fluorine, chlorine, bromine or iodine. In one embodiment, the halogen is preferably chosen from fluorine and chlorine.

The "R-M-X-CO-" group is most preferably chosen from p-chloro-cinnamoyl, p-hydroxy-cinnamoyl, p-methyl-cinnamoyl, 2,4-di-fluoro-cinnamoyl, 3-fluoro-4-chloro-cinnamoyl, 3-chloro-4-fluoro-cinnamoyl, 4-fluoro-cinnamoyl, 3-fluoro-cinnamoyl, 3,4-di-fluoro-cinnamoyl, 2-quinoline-acyl, 2-thienyl-acryloyl, 2-nitro-4-chloro-benzoyl and 2-naphthyl-oxy-acetyl.

The pharmaceutically acceptable salt of the conjugates disclosed above is part of the invention, the basic nitrogen atoms in the molecules of the conjugates in the present invention can form salts with acid, not be particularly limited, with any pharmaceutically acceptable acid such as inorganic acids, including, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and organic acids, including, for example, oxalic acid, fumaric acid, maleic acid, succinic acid, citric acid, tartaric acid, methanesulfonic acid and p-toluenesulfonic acid, etc.

The conjugates of muramyl dipeptide analogue and paclitaxel, or muramyl dipeptide analogue and docetaxel, and salts thereof can be synthesized by the general and exemplary methods as follows:

1. Paclitaxel-2'-O-alkane-di-acid monoester or docetaxel-2'-O-alkane-di-acid monoester are synthesized by liquid-phase synthesis;

2. Muramyl dipeptide analogue (MDA) is synthesized by solid-phase or liquid-phase synthesis;

3. Conjugates of muramyl dipeptide analogue and paclitaxel, or muramyl dipeptide analogue and docetaxel are synthesized by liquid-phase synthesis.

The method for preparing paclitaxel-2'-O-alkane-di-acid monoester or docetaxel-2'-O-alkane-di-acid monoester through liquid-phase synthesis comprises the steps as follows:

1) Preparation of the paclitaxel-2'-O-alkane-di-acid monoester through liquid-phase synthesis

(1) Paclitaxel, alkane-di-anhydride and 4-N,N-dimethyl pyridine (DMAP) are dissolved in pyridine, and are stirred for 4 h at room temperature (r.t);

20

(2) The solution of step (1) is diluted with ethylacetate (AcOEt), the AcOEt layer is washed with saturate $CuSO_4$ solution and H_2O sequentially;

(3) At last, the AcOEt layer is separated and then concentrated under vacuum, abundant water is added into the residue, white solid precipitated, the paclitaxel-2'-O-alkane-di-acid monoester was obtained as white solid after filtration and lyophilization.

2) Preparation of the docetaxel-2'-O-alkane-di-acid monoester through liquid-phase synthesis

(1) Docetaxel, alkane-di-anhydride and 4-N,N-dimethyl pyridine are dissolved in N,N-dimethylformamide (DMF), and are stirred for 2 h at r.t;

(2) The DMF solution is diluted with dichloromethane (DCM), then, the DCM layer is washed with HCl aqueous solution (2N) and H_2O sequentially;

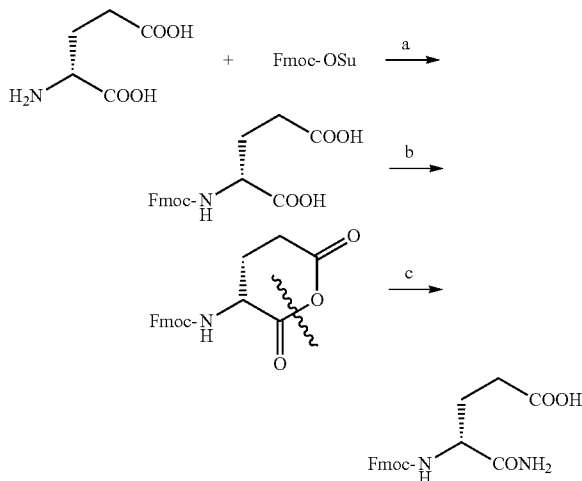
(3) At last, the DCM layer is separated and concentrated under vacuum, the residue is dissolved in a little methanol, then abundant water is added into the residue, white solid precipitated, docetaxel-2'-O-alkane-di-acid monoester is obtained as white solid after filtration and lyophilization.

The method for preparing the muramyl dipeptide analogue through solid-phase synthesis and liquid-phase synthesis comprises the steps as follows:

1) Solid-Phase Synthesis:

(1) Synthesis of amino acid intermediate Fmoc-D-iso-Gln-OH;

The route is shown below:

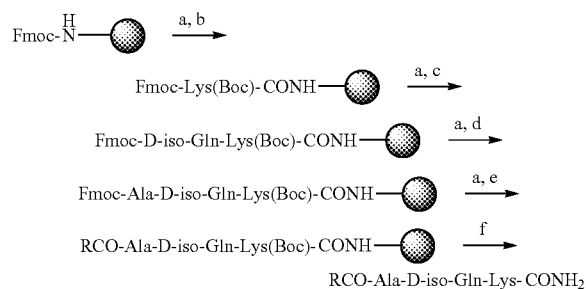


Reagents and conditions: (a) r.t, 3 d; (b) dicyclohexyl carbodiimide (DCC), $0^\circ C$, 5 h, r.t, 20 h; (c) NH_3 ; $-10^\circ C$, 1.5 h.

(2) Then, by employing any one of amino resin such as Rink-Amide AM (loading 0.88 mmol/g) as carrier of solid phase, Fmoc-L-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-L-Ala-COOH and carboxylic acid are introduced to the resin by solid-phase synthesis; After the condensation reaction is completed, the muramyl dipeptide analogue is obtained by steps, such as washing the resin thoroughly, cleaving the crude product from the resins, and purifying the crude product, etc. Acylation involved herein are conventional amide condensation reaction, the condensation reaction is completed by adding the excess amount of reagents (such as amino acid or carboxylic acid) and superactive condensing agent (such as 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,

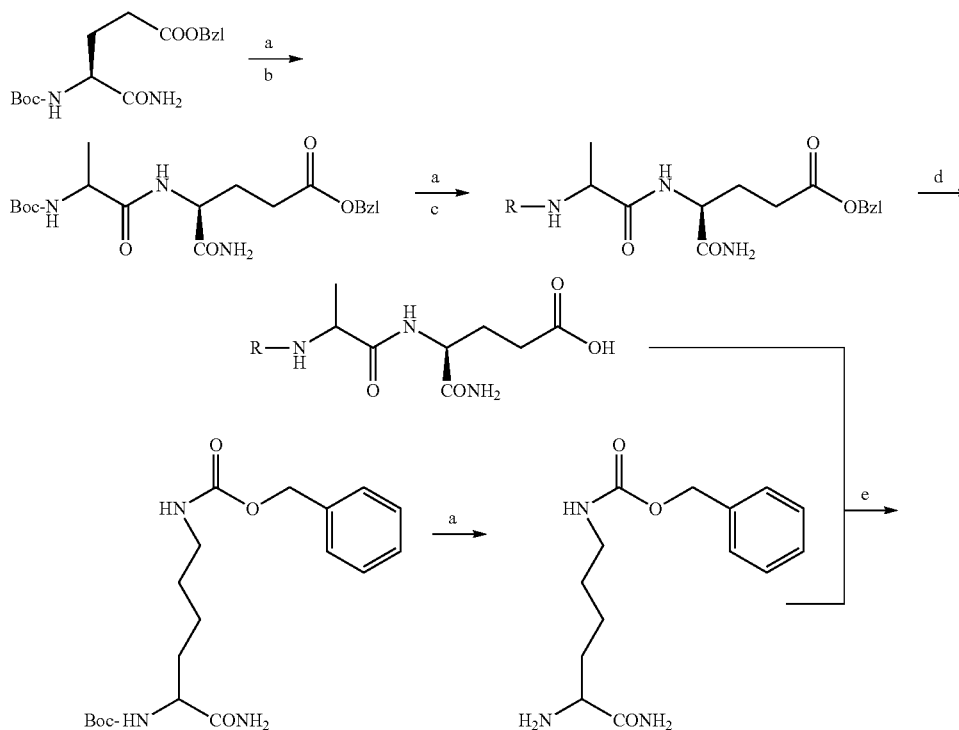
21

3,3-tetramethyluronium hexafluorophosphate (HATU), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP), or Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP). The characteristic of the method is that the introduction of the carboxylic acid is not affected by structure (such as aromatic and non-aromatic, straight chain and branched chain), the steric hindrance, physicochemical property, electronic effect, the ring system and the line system, etc., So the three amino acids above can be replaced by any natural or unnatural amino acid, such as Fmoc-D-Lys(Boc)-COOH, Fmoc-L-iso-Gln-COOH, Fmoc-L-Gln-COOH, Fmoc-D-Gln-COOH or Fmoc-D-Ala-COOH. The route is shown as below:



= Rink Amide-Am Resin

Reagents and conditions: (a) 20% piperidine/DMF; r.t, 1 h; (b) Fmoc-Lys(Boc)-OH, HOBT, N,N'-Diisopropyl carbodiimide (DIC); r.t, 8 h; (c) Fmoc-D-iso-Gln-OH, HOBT, DIC; r.t, 12 h; (d) Fmoc-Ala-OH, HOBT, DIC; r.t, 8 h; (e) organic acid, HOBT, DIC; r.t, 8 h; (f) 90% Trifluoroacetic acid(TFA)/H₂O, r.t, 2 h.

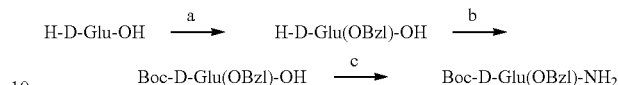


22

2) Liquid-Phase Synthesis:

(1) Synthesis of amino acid intermediate Boc-D-Glu(Obzl)-NH₂;

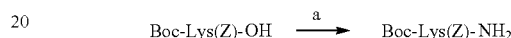
The route is shown below:



Reagents and conditions: (a) C₆H₅CH₂OH, BF₃·Et₂O; r.t, 15 h; (b) (Boc)₂O, NaHCO₃; r.t, 20 h; (c) HOSu, DCC, NH₃; -10° C., 1.5 h.

(2) Synthesis of amino acid intermediate Boc-Lys(Z)-NH₂;

The route is shown below:



Reagents and conditions: (a) HOSu, DIC, NH₃; -10° C., 1.5 h.

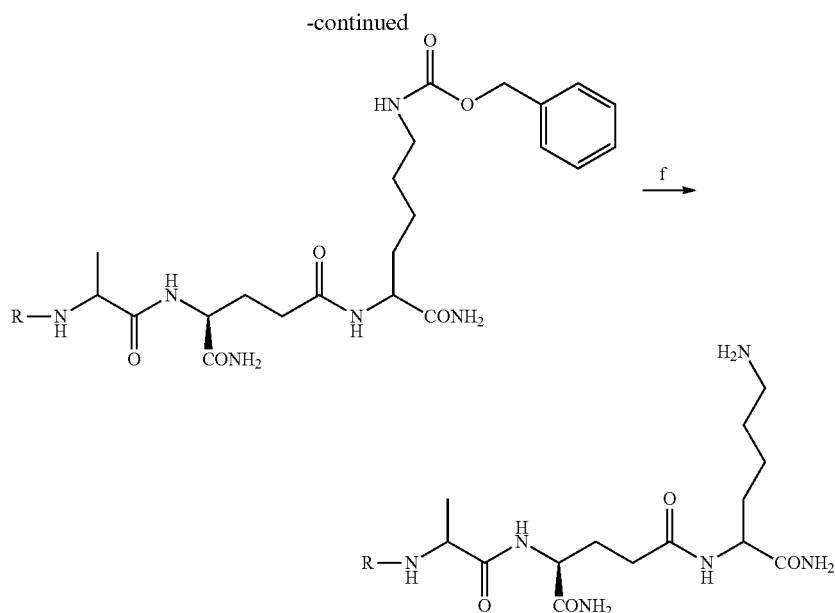
(3) Then, the dipeptide fragment Boc-Ala-D-Glu(Obzl)-NH₂ and the tripeptide fragment R-Ala-D-Glu(Obzl)-NH₂ are synthesized by the active ester method, and the protecting group Bzl in tripeptide is removed by using hydrobromic acid in acetic acid solution or under other feasible acid/basic conditions, the tetrapeptide R-Ala-D-iso-Gln-Lys(Z)-NH₂ is synthesized by the active ester method;

(4) At last, the protecting group Z is removed by using the mixture of boron trifluoride ethylether, TFA and ethanethiol (V/V/V=9:9:2) to obtain the crude product, and muramyl dipeptide analogue is obtained after purification.

The route is shown below:

23

24



Reagents and conditions: (a) 50% TFA/DCM; r.t. 1 h; (b) Boc-Ala-OH, HOSu, DIC; 0° C., 5 h, r.t., 20 h; (c) organic acid ©, HOSu, DIC; 0° C., 5 h, r.t., 20 h; (d) HBr/HOAc; r.t., 3 h; (e) HOSu, DIC; 0° C., 5 h, r.t., 20 h; (f) BF₃.Et₂, TFA, EtSH (9:9:2); r.t. 2 h.

The method for preparing the conjugates of muramyl dipeptide analogue and paclitaxel, or muramyl dipeptide analogue and docetaxel comprises the steps as follows:

1) First, paclitaxel-2'-O-alkane-di-acid monoester or docetaxel-2'-O-alkane-di-acid monoester, HOSu and DIC with certain molar ratio (2:1-1:2) are dissolved in dimethyl sulfoxide (DMSO) or DMF or N-methyl pyrrolidone, etc., the resulting solution is reacted for 1-10 hours at the temperature of -20° C. to 50° C.;

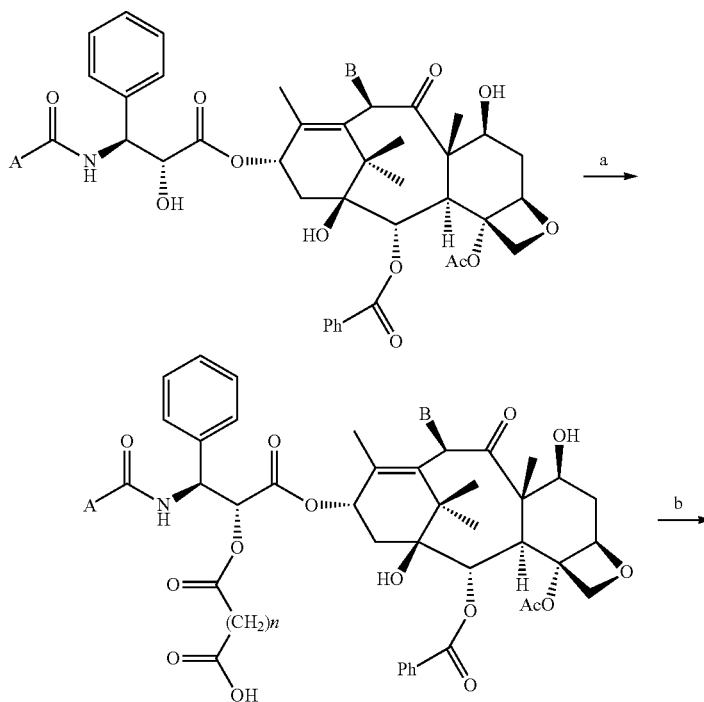
2) Then, the muramyl dipeptide analogue with mole numbers equal to that of paclitaxel-2'-O-alkane-di-acid monoester or

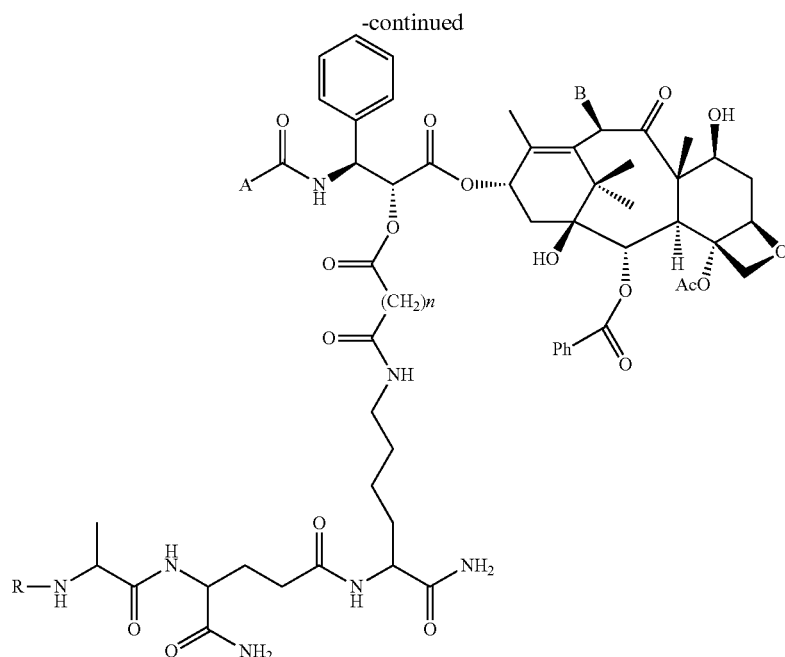
docetaxel-2'-O-alkane-di-acid monoester is added to the solution of DMSO or DMF or N-methyl pyrrolidone, etc., the pH of the reaction system is adjusted to 6-8 by alkalescence reagent such as N-methyl morpholine, etc., the reaction is continued for 1-10 hours, the conjugate is obtained after reaction completed;

3) At last, any one solvent selected from water, methanol, ethanol, diethyl ether, petroleum ether, ethyl butyl ether is added to the reaction solution, and the solid precipitated is filtered, the crude product is purified to obtain the target product;

4) The method for purification includes preparative HPLC and recrystallization.

The route is shown as below:





Reagents and conditions: (a) alkane-di-anhydride, DMAP, r.t, 4 h; (b) HOSu, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDC-HCl), DMSO, r.t, 20 h; MDA (muramyl dipeptide analogue) derivatives, r.t, 12 h.

The alkane diacid is chosen from C_4 - C_{14} alkane diacid, the alkane dianhydride is chosen from C_4 - C_{14} alkane dianhydride.

The method for preparing the conjugates as disclosed in the present invention has mild reaction condition, short reaction time, stable yield, so that it is suitable for building compound library through, for example, combinatorial chemistry method, which also belong to the claim scope of the present invention.

People skilled in the art may adjust the steps mentioned above to improve the yield, they may design mutants based on the basic knowledge of the field, such as selecting the reactant, solvent and temperature. Also, they can, by using a variety of conventional protecting groups, avoid side reaction and thus increase the yield. These common reactions may be referenced in books on peptide synthesis chemistry such as 1) Gang LIU and Kit S. LAM, "One-bead one-compound combinatorial library method", *Combinatorial Chemistry, A Practical Approach*, Edited by Hicham Fenniri, OXFORD University Press, 2000, Chapter 2, pp 33-50; 2) Gang Liu, Xiaoyi Xiao, et al. *Looking for combinatorial chemistry in drug research*, Science Press, 2003, 6; 3) N. Leo Benoiton, *Chemistry of Peptide Synthesis*, published in 2005 by CRC press; 4) Miklos Bodanszky, *Principles of Peptide Synthesis* by Publisher of Springer Verlag (Edition: 2ND/REV). Such modifications or changes are within the scope of the present invention.

The conjugates disclosed in the present invention can be used in preparation of medicament for preventing and/or treating cancer. The cancer can be chosen from melanoma, gastric cancer, lung cancer, breast cancer, renal cancer, liver cancer, oral cavity epidermal carcinoma, cervical cancer, oophoroma, pancreatic cancer, prostatic cancer and colonic cancer.

The present invention therefore also relates to compositions comprising therapeutic amount of conjugate(s) disclosed in the present invention, and one or more pharmaceutically acceptable carriers and/or excipients. The pharmaceutically acceptable carriers include, for example, saline, buffered saline, dextrose, water, glycerol, ethanol, hereinafter discussed in more detail. If desired, the composition can also comprise a smaller amount of wetting or emulsifying agent(s), or pH buffering agent(s). The composition can be liquid solution, suspension, emulsion, tablets, pills, capsules, sustained release preparations or powders. The composition can be suppositories using traditional binders and carriers such as tricarboxylic acid glyceride. Oral preparation can use standard carriers such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose and magnesium carbonate et al, in pharmaceutical grade. As required by different preparations, the related preparation may involve mixing, granulating and compressing or dissolving the active ingredients. Also, the composition may be prepared into nanoparticles.

The pharmaceutically acceptable carrier used herein can be solid or liquid.

The carrier or excipient can be a delayed-release material known to those skilled in the art, such as glyceryl monostearate or glyceryl distearate, and can also include waxes, ethyl cellulose, hydroxypropyl methyl cellulose, and methylmethacrylate etc. The recognized PHOSALPG-50 (phospholipid with 1,2-propanediol was concentrated, A. Nattermann & Cie. GmbH) in 0.01% Tween-80 used for the preparation of acceptable oral preparation of other conjugates, can be also employed in preparation of conjugates disclosed in the present invention.

Conjugates disclosed in the present invention can be administered in variety of pharmaceutical forms. If solid carrier is employed, the preparation can be tablet, hard capsule with powder or small pills in it, lozenge or sugar lozenge form. The amount of solid carrier can be widely ranged, but preferably from about 25 mg to about 1 g. If a liquid carrier is used, the preparation can be syrups, emulsions, soft gelatin

27

capsules, sterile injectable solution or suspension or non-aqueous liquid suspension in the ampoule or vial.

Various release systems are known and can be used for the administration of conjugates or various preparations thereof, these preparations include tablets, capsules, injectable solutions, liposome capsules, microparticles, microcapsules etc. The method introduced includes but not limited to dermal, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, pulmonary, epidural, ophthalmic and oral (preferred) administration. Conjugates can be administered through any convenient or suitable route, for example, injection or bolus injection, absorption through epithelial or mucosal route (e.g., oral mucosa, rectal and intestinal mucosa, etc.) or drug elution stent, or can be administered together with other biologically active agents, or can be administered systemically or locally. For treatment or prevention of nasal, bronchial or pulmonary diseases, the preferred route of administration is oral, nasal, or bronchial aerosol or nebulizer.

BRIEF DESCRIPTION OF DRAWINGS

FIGS. 1A and 1B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MTC-220 in 60 human origin tumor lines.

FIGS. 2A and 2B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MTC-302 in 60 human origin tumor lines.

FIGS. 3A and 3B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MTC-213 in 60 human origin tumor lines.

FIGS. 4A and 4B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MTC-219 in 60 human origin tumor lines.

FIGS. 5A and 5B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MTC-233 in 60 human origin tumor lines.

FIGS. 6A and 6B The 50% growth inhibition (GI_{50}) and 50% lethal concentration (LC_{50}) of MDC-400 in 60 human origin tumor lines.

FIG. 7 Anti-tumor activities of MTC-301, 302, 303 and 304 in 10 tumor cell lines in vitro.

FIG. 8 Anti-tumor activities of MTC-305, 306, 307 and 308 in 10 tumor cell lines in vitro.

FIG. 9 Anti-tumor activities of MDC-403, 404 and 405 in 10 tumor cell lines in vitro.

FIG. 10 Anti-tumor activities of MDC-406, 407 and 408 in 10 tumor cell lines in vitro.

FIG. 11 The effect on body weight of MTC-220 in MDA-MB-231 tumor bearing mice.

FIG. 12 The growth inhibition of MTC-220 in MDA-MB-231 tumor bearing mice.

FIG. 13 The effect on RTV of MTC-220 in MDA-MB-231 tumor bearing mice which was treated with a same dose by different administration method.

FIG. 14 The effect on body weight of MTC-220 in MDA-MB-231 tumor bearing mice which was treated with a same dose by different administration method.

FIG. 15 The effect on body weight of MTC-220 in H460 tumor bearing mice.

FIG. 16 The growth inhibition of MTC-220 in H460 tumor bearing mice.

FIG. 17 The growth inhibition of MTC-220 in MCF-7 tumor bearing mice.

FIG. 18 The effect on body weight of MTC-220 in MCF-7 tumor bearing mice.

28

FIG. 19 The growth inhibition of MTC-220 in A549 tumor bearing mice.

FIG. 20 The effect on body weight of MTC-220 in A549 tumor bearing mice.

FIG. 21 The effect on body weight of MTC-220 in H1975 tumor bearing mice.

FIG. 22 The growth inhibition of MTC-220 in H1975 tumor bearing mice.

FIG. 23 The growth inhibition of MTC-220 in breast cancer mice (1).

FIG. 24 The effect on body weight of MTC-220 in breast cancer mice (2).

FIG. 25 Anti-tumor natural metastasis activities of MTC-220 in breast cancer mice (3).

FIG. 26 The growth inhibition activity of MTC-220 in Lewis lung cancer mice (1).

FIG. 27 The effect on body weight of MTC-220 in Lewis lung cancer mice (2).

FIG. 28 Anti-tumor natural metastasis activities of MTC-220 in Lewis lung cancer mice (3).

FIG. 29 Anti-tumor artificial metastasis activities of MTC-220 in Lewis lung cancer mice.

DETAILED EXAMPLES

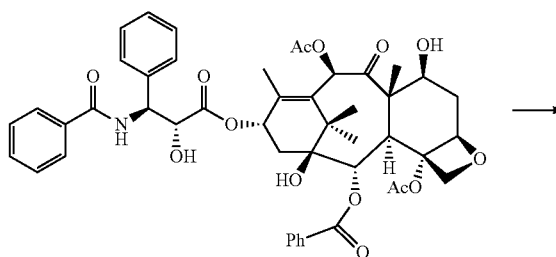
The present disclosure is further illustrated by the following examples of synthesis of conjugates of Muramyl Dipeptide Analogue and paclitaxel, or of Muramyl Dipeptide Analogue and docetaxel and biological experiments thereof. Those skilled in the art should understand that these examples are merely for illustrative purposes, without limiting the scope of the present invention. The scope of the present invention is limited only by the claims. Under conditions without departing from the scope of the claims, people skilled in the art can modify or improve aspects of the present invention, such modifications and improvements also belong to the scope of protection of the present invention.

Also, unless otherwise specified, materials and the reagents used in the following examples are those commonly used in the field, which can be commercially available; the intermediates used can be commercially available or prepared by known methods; methods used are conventional methods known by those skilled in the art.

Example 1

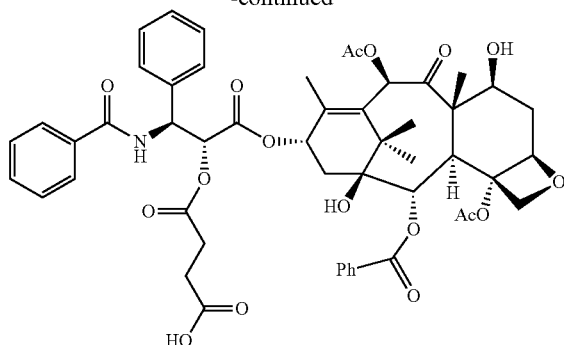
Liquid-phase Synthesis of Paclitaxel 2'-O-succinic acid monoester (Synthetic method refer to CN200510081265)

Synthetic route was shown below



29

-continued



Reagents and conditions: succinic anhydride, DMAP, r.t, 4 h.

8.53 g (1.0 eq) Paclitaxel, 1.2 g (1.2 eq) succinic anhydride, 0.12 g (0.1 eq) 4-N,N-dimethyl pyridine were dissolved in pyridine, then stirred at r.t for 4 h. After the reaction completed, the pyridine solution was diluted with AcOEt. And then, the AcOEt layer was washed with saturated aqueous CuSO₄ solution, and H₂O sequentially. At last, the AcOEt layer was separated. The AcOEt solution was concentrated under vacuum, and then abundant water was added into the residue, white solid precipitated in the system. After filtration and lyophilization, 8.1 g target product was obtained with a yield of 85%, m.p.=178~180° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.63 (1H, br.s, 1-OH), 5.40 (1H, d, J=8.4 Hz, 2-H), 3.58 (1H, d, J=8.4 Hz, 3-H), 4.90 (1H, d, J=10.8 Hz, 5-H), 1.62 (1H, t, J=14.4 Hz, 6-H_a), 2.31 (1H, m, 6-H_b), 4.10 (1H, dd, J=12.0 and 8.4 Hz, 7-H), 4.89 (1H, d, J=10.8 Hz, 7-OH), 6.29 (1H, s, 10-H), 5.81 (1H, t, J=10.8 Hz, 13-H), 1.51 (1H, m, 14-H_a), 1.81 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 1.02 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.49 (3H, s, 19-H), 3.98 (1H, d, J=10.2 Hz, 20-H_a), 4.02 (1H, d, J=10.2 Hz, 20-H_b), 2.10 (3H, s, 4-OCOCH₃), 2.23 (3H, s, 10-OCOCH₃), 5.35 (1H, d, J=10.8 Hz, 2'-H), 5.54 (1H, dd, J=10.8 and 10.2 Hz, 3'-H), 9.21 (1H, d, J=10.2 Hz, 3'-NH), 7.49 (2H, m, ph-o-H), 7.47 (2H, m, ph-m-H), 7.54 (1H, m, ph-p-H), 7.84 (2H, d, J=10.2 Hz, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.19 (1H, m, NBz-p-H), 7.97 (2H, d, J=9.6 Hz, OBz-o-H), 7.65 (2H, m, OBz-m-H), 7.72 (1H, m, OBz-p-H), 2.61 (2H, t, J=7.2 Hz, —CH₂—CH₂—COOH), 2.32 (2H, m, —CH₂—CH₂—COOH), 12.23 (1H, br.s, —CH₂—CH₂—COOH).

¹³C-NMR (150 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.3 (8-C), 202.3 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.4 (14-C), 42.9 (15-C), 26.3 (16-C), 21.3 (17-C), 13.8 (18-C), 9.7 (19-C), 75.2 (20-C), 169.6 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.7, 20.6 (10-OCOCH₃), 169.0 (1'-C), 74.7 (2'-C), 53.9 (3'-C), 166.4 (3'-NHCO), 137.3 (ph-q-C), 127.6 (ph-o-C), 128.3 (ph-m-C), 131.4 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 128.6 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.5 (OBz-o-C), 128.6 (OBz-m-C), 133.4 (OBz-p-C), 172.9, 28.4, 30.9, 171.6 (—CO—CH₂—CH₂—COOH).

IR: 3471.3 (ν_{OH} and ν_{NH}), 3065.2 (ν_{C—H}), 2957.5 (ν_{C—H}), 1717.3, 1642.0 (ν_{C=O}), 1602.4, 1579.8, 1525.9 (ν_{C=C}), 1487.4, 1370.4 (δ_{C—H}), 1241.4 (ν_{C—O—C}), 978.6, 904.7, 948.5, 776.0, 708.3 (δ_{C—H}).

ESI-MS: 954.75 [M+H]⁺, 1929.13 [2M+Na]⁺.

HR-MS(TOF): 954.3552 [M+H]⁺, 976.3352 [M+Na]⁺, C₅₁H₅₅NO₁₇.

30

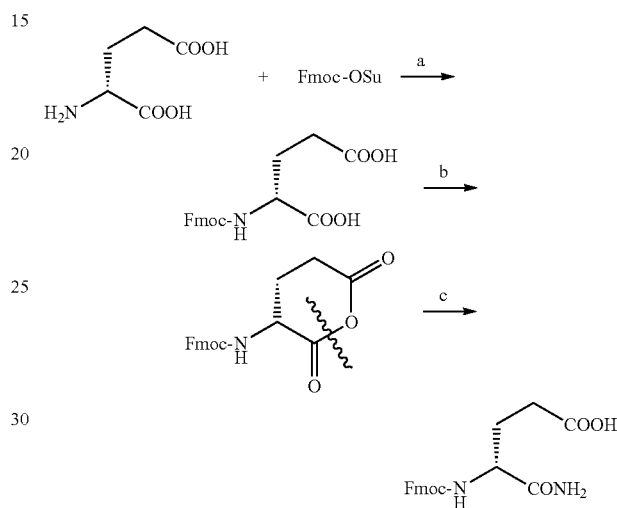
Example 2-3

Solid-Phase Synthesis of Muramyl dipeptide Analogue MDA

Example 2

Synthesis of Fmoc-D-iso-Gln-OH

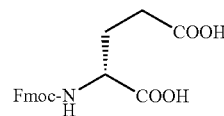
Synthetic route was shown below



Reagents and conditions: (a) r.t, 3 d; (b) DCC, 0° C., 5 h, r.t, 20 h; (c) NH₃; -10° C., 1.5 h.

Steps 1

Synthesis of Fmoc-D-Glu-OH

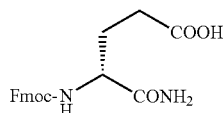


In an ice-water bath, a solution of D-glutamic acid (H-D-Glu-OH, 29.4 g, 1.0 eq) in a mixture of acetone and H₂O (V/V=1:1) was stirred. After the solid was fully dissolved, NaHCO₃ (23.3 g, 1.1 eq) was added in portions, then Fmoc-OSu (67.4 g, 1.0 eq) were added slowly and the reaction was stirred for additional 3 days at r.t. The mixture was then cooled in ice-water bath again, and pH was adjusted to 2-3 with 2.0N HCl. After removal of acetone under reduced pressure, the remaining solution was extracted with AcOEt (400 mL×4). The organic layer was separated and combined, dried with MgSO₄ overnight, and concentrated to a small volume under reduced pressure. Then residue was recrystallized with ethylacetate-cyclohexane system. After filtration, 59.8 g of target product was obtained as a white solid with a yield of 81%.

31

Steps 2

Synthesis of Fmoc-D-iso-Gln-OH



Fmoc-D-Glu-OH (59.8 g, 1.0 eq) was dissolved in anhydrous tetrahydrofuran (THF) (324 mL). DCC (40.1 g, 1.2 eq) was then added while stirring in ice-water bath. The reaction mixture was allowed to warm to r.t and stirring was maintained for additional 8 h to produce 1,3-dicyclohexylurea (DCU). The precipitates were filtered off, and washed with small amount THF. Dry ammonia gas was then bubbled through the filtrate which was stirred in a NaCl salt-ice bath. The reaction was completed after 1.5 h when no more white solid was precipitated. Still standing for 30 min, small amount MeOH was added to dissolve the solid. The mixture was cooled in an ice-water bath again. Then 2.0 N HCl was added carefully and slowly to adjust pH to 2-3. The solvent was evaporated under vacuum. The resulting solid was dissolved in AcOEt and then washed with diluted HCl, saturated aqueous NaHCO₃ solution, and H₂O sequentially. The organic layer was separated and combined, then dried with MgSO₄ overnight, filtered and evaporated under vacuum. Then residue was recrystallized with ethylacetate-cyclohexane system. After filtration, 46.5 g target product was obtained with a yield of 78%. m.p.=204~205° C., [α]_D²⁰=-4.2° (C=10 mg/mL, DMF).

¹H-NMR (500 MHz, DMSO): 7.88 (2H, d, J=8.0 Hz), 7.72 (2H, m), 7.42 (2H, m), 7.40 (1H, m), 7.40 (1H, br.s), 7.32 (2H, m), 7.02 (1H, br.s), 4.27 (2H, m), 4.20 (1H, m), 3.93 (1H, dd, J=13.5 and 8.5 Hz), 2.25 (2H, m), 1.89 (1H, m), 1.73 (1H, m).

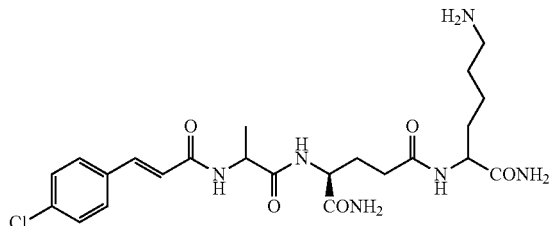
¹³C-NMR (125 MHz, DMSO): 173.9, 173.4, 155.9, 143.8, 140.7, 127.6, 127.0, 125.3, 120.0, 65.6, 53.8, 46.6, 30.4, 27.2.

ESI-MS: 369.03 [M+H]⁺, 759.98 [2M+Na]⁺.

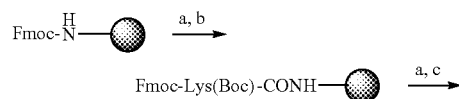
HR-MS(TOF): 369.1448 [M+H]⁺, 759.2623 [2M+Na]⁺, C₂₀H₂₀N₂O₅.

Example 3

Solid-Phase Synthesis of Muramyl Dipeptide Analogue

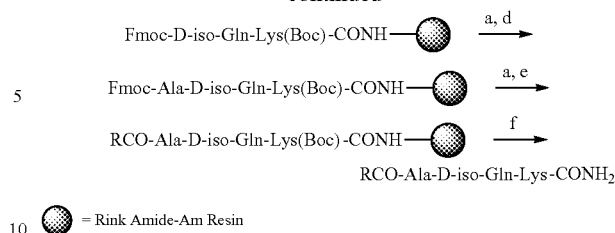


Synthetic route was shown below



32

-continued



Reagents and conditions: (a) 20% piperidine/DMF; r.t, 1 h; (b) Fmoc-Lys(Boc)-OH, HOBt, DIC; r.t, 8 h; (C) Fmoc-D-iso-Gln-OH, HOBt, DIC; r.t, 12 h; (d) Fmoc-Ala-OH, HOBt, DIC; r.t, 8 h; (e) 4-chloro-cinnamic acid (R), HOBt, DIC; r.t, 8 h; (f) 90% TFA/H₂O, r.t, 2 h.

100.0 g Rink-amide-AM resin (loading 0.88 mmol/g, 1.0 eq) was put into a solid-phase reactor and vacuumed under reduced pressure for 1 h. Anhydrous DCM (500 mL) was added to swell the resin for 45 min and then removed. The Fmoc group of resin was removed by using of 20% (Volume percentage) piperidine/DMF for 1 h at r.t, followed by drainage of the liquid phase. The resin was washed thoroughly with DMF (500 mL×6) and DCM (500 mL×6) respectively. Fmoc-Lys(Boc)-COOH (61.8 g, 1.5 eq), HOBt (17.8 g, 1.5 eq), DIC (20.8 mL, 1.5 eq) and DMF (500 mL) were added into the reactor to introduce the first amino acid, which was bonded to the resin after reacting for 8 h at r.t. When it was negative by the ninhydrin method, the coupling reaction was completed. The liquid phase was removed, and the resin was thoroughly washed with DMF (500 mL×6) and DCM (500 mL×6) respectively. Then the Fmoc was removed by using 20% (Volume percentage) piperidine/DMF. Fmoc-D-iso-Gln-OH (48.5 g, 1.5 eq), HOBt (17.8 g, 1.5 eq), DIC (20.8 mL, 1.5 eq), and DMF (500 mL) were added to introduce the second amino acid to the solid phase. The reaction was lasted 12 h and was monitored by ninhydrin method. When ninhydrin test indicted the reaction was complete, the liquid phase was removed, 500 mL 20% (Volume percentage) piperidine/DMF was added to remove Fmoc, removed the liquid phase again after 1 h, the resin was washed with DMF (500 mL×6) and DCM (500 mL×6) respectively. Fmoc-Ala-COOH (41 g, 1.5 eq), HOBt (17.8 g, 1.5 eq), DIC (20.8 mL, 1.5 eq) and 500 mL DMF were added to introduce the third amino acid. The reaction was lasted 12 h and was monitored by ninhydrin method. When ninhydrin test indicted the reaction was complete, the liquid phase was removed, 500 mL 20% (Volume percentage) piperidine/DMF was added to remove Fmoc, liquid phase was removed again after 1 h, the resin was washed with DMF (500 mL×6) and DCM (500 mL×6) respectively. Chlorocinnamic acid (24.1 g 1.5 eq), HOBt (17.8 g, 1.5 eq), DIC (20.8 mL, 1.5 eq) and 500 mL DMF were added to introduce the organic acid. The reaction was lasted 8 h and was monitored by ninhydrin method. When ninhydrin test indicted the reaction was complete, the liquid phase was removed, the resin was washed with DMF (500 mL×6) and DCM (500 mL×6) respectively. TFA water solution 90% (Volume percentage) was added to the reactor, the reaction was lasted for 2 h. Collected the liquid phase, another TFA water solution 90% (Volume percentage) was added to the reactor, the reaction was lasted for 2 h, collected the liquid phase again, the resin was washed with 200 mL DCM. TFA water solutions and DCM were combined and evaporated under vacuum. In ice bath, to the residue was added abundant diethylether, white solid precipitated, removed the supernatant. The white solid was grinded and washed with diethylether for several times, filtration gave crude product (39.8) with the yield 89%. The crude product was purified by ODS column chromatography with gradient elution, methanol/wa-

33

ter to produce 35.88 g target product in 98.5% purity. m.p.=215~217° C., $[\alpha]_D^{25}+37.7^\circ$ (C=11.05 mg/mL, DMF).

¹H-NMR (600 MHz, DMSO-d₆): 7.47 (2H, d, J=8.4 Hz, 2 and 6-H), 7.57 (2H, d, J=8.4 Hz, 3 and 5-H), 7.39 (1H, d, J=15.9 Hz, 7-H), 6.75 (1H, d, J=15.9 Hz, 8-H), 8.39 (1H, d, J=6.6 Hz, 10-H), 4.38 (1H, m, 11-H), 1.26 (3H, m, 12-H), 8.21 (1H, d, J=8.4 Hz, 14-H), 4.14 (1H, m, 15-H), 6.98 (1H, s, 17-H_a), 7.41 (1H, s, 17-H_b), 1.71 (1H, m, 18-H_a), 1.97 (1H, m, 18-H_b), 2.15 (2H, t, J=7.2 Hz, 19-H), 7.90 (1H, d, J=8.4 Hz, 21-H), 4.11 (1H, m, 22-H), 7.10 (1H, s, 24-H_a), 7.30 (1H, s, 24-H_b), 1.46 (1H, m, 25-H_a), 1.63 (1H, m, 25-H_b), 1.27 (2H, m, 26-H), 1.53 (2H, m, 27-H), 2.73 (2H, m, 28-H), 7.75 (2H, br.s, 29-H).

¹³C-NMR (150 MHz, DMSO-d₆): 134.0 (1-C), 129.0 (2 and 6-C), 129.2 (3 and 5-C), 133.8 (4-C), 137.6 (7-C), 122.7 (8-C), 164.7 (9-C), 48.8 (11-C), 18.1 (12-C), 172.4 (13-C), 52.2 (15-C), 173.8 (16-C), 27.7 (18-C), 31.7 (19-C), 171.6 (20-C), 52.1 (22-C), 173.3 (23-C), 31.3 (25-C), 22.4 (26-C), 26.8 (27-C), 38.7 (28-C).

IR: 3282.3, 3202.2 (ν_{OH} and ν_{NH}), 3067.3 (ν_{CH}), 2938.0 (ν_{CH}), 1609.5 (ν_{C=O}), 1537.5, 1450.2 (ν_{C=C}), 1199.0, 1180.2, 1130.6 (δ_{CH}), 972.4, 820.4, 799.4, 720.0 (δ_{CH} and ν_{C-Cl}).

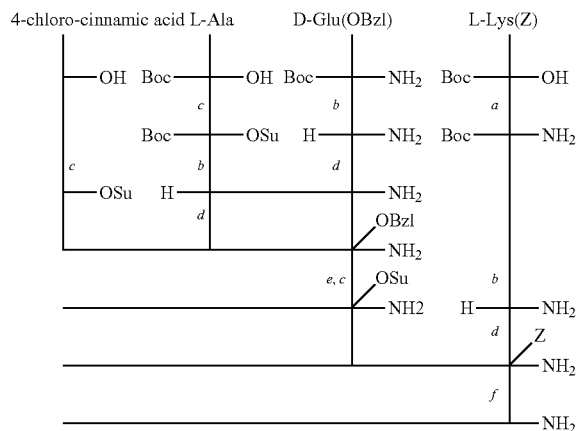
ESI-MS: 509.60 [M+H]⁺, 1017.24 [2M+H]⁺.

HR-MS(TOF): 509.2292 [M+H]⁺, C₂₃H₃₃ClN₆O₅.

Example 4-10

Liquid-Phase Synthesis of Muramyl Dipeptide Analogue MDA

The synthetic route was shown below



Reagents and conditions: (a) HOSu, DIC, NH₃; -10° C., 1.5 h; (b) 50% TFA/DCM; r.t. 1 h; (c) HOSu, DIC; 0° C., 5 h, r.t. 20 h; (d) 0° C., 5 h, r.t. 24 h; (e) HBr/HOAc; r.t. 3 h; (f) BF₃.Et₂O, TFA, EtSH (9:9:2); r.t. 2 h.

Example 4

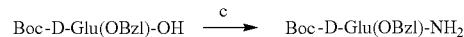
Liquid-phase synthesis of Boc-D-Glu(OBzl)-NH₂

The synthetic route was shown as below:



34

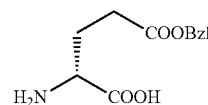
-continued



Reagents and conditions: (a) C₆H₅CH₂OH, BF₃.Et₂O; r.t. 15 h; (b) (Boc)₂O, NaHCO₃; r.t. 20 h; (c) HOSu, DCC, NH₃; -10° C., 1.5 h.

Step 1

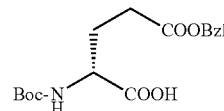
Liquid-phase synthesis of H-D-Glu(OBzl)-OH



To a solution of 29.1 g (1.0 eq) H-D-Glu-OH in 205.6 mL (10.0 eq) benzyl alcohol which was stirred at r.t. 47.7 mL (2.0 eq) boron trifluoride etherate solution was added slowly, and 10 min later, all of the substrate was dissolved. The reaction was completed in 15 h, 616.8 mL (3 times of the volume of benzyl alcohol) THF was added, stirred and 55.1 mL (2.0 eq) triethylamine was added slowly. A large number of white viscous precipitate precipitated. The THF was removed under reduced pressure; the residue was cooled, after adding the proper amount of the AcOEt, the viscous precipitate turned to powder. 36.6 g target compound was obtained with yield of 78% after filtration and drying. m.p.=174~176° C.

Step 2

Liquid-phase synthesis of Boc-D-Glu(OBzl)-OH

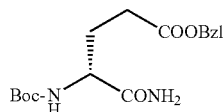


36.6 g (1.0 eq) H-D-Glu(OBzl)-OH was dissolved in 500 mL dioxane/water (v/v=1:1), 67.3 g (2.0 eq) Boc anhydride and 25.3 g sodium dicarbonate (2.0 eq) were added sequentially; and an oil bath heating was employed for dissolving all the substrates. The solution was stirred at r.t. for 20 hours. After the completion of the reaction, the dioxane was removed under vacuum, and large number of viscous precipitate was obtained. The precipitate was diluted with 500 mL water, and stirred for another 30 minutes to fully dissolution. The pH of the solution was adjusted to 2~3 by 2 N HCl aqueous solution in ice bath, and the mixture became muddy, and was allowed to stand for 30 minutes.

The solution was extracted with AcOEt for 5 times, and the organic phase was combined, dried with MgSO₄ overnight. After filtration, the AcOEt was removed under vacuum, and 48.6 g yellow oily target compound was obtained with yield of 86%.

35

Step 3

Liquid-phase synthesis of Boc-D-Glu(OBzl)-NH₂

48.6 g (1.0 eq) Boc-D-Glu(OBzl)-OH was dissolved in tetrahydrofuran, 24.8 g (1.5 eq) HOSu and 44.5 g (1.5 eq) DCC were added sequentially. After stirring for 5 hours in ice bath, the reaction was warmed to r.t and stirred for another 20 hours. A large number of white precipitate (DCU) precipitated, the precipitate was filtered out and washed with little tetrahydrofuran. The filtrate was stirred in ice-salt bath, and anhydrous ammonia was introduced to the solution. After 15 minutes, a large number of white precipitate precipitated, and stirred the mixture for another 1.5 hours, no more white solid precipitated out, and the reaction was completed. The precipitate was filtered and washed with tetrahydrofuran, and yellow oil was obtained after removing the tetrahydrofuran filtrate under vacuum. The yellow oil was diluted with AcOEt; and the pH of the solution was adjusted to 7 with 2N HCl aqueous solution in ice bath, and the solution was allowed to stand for 30 minutes. The AcOEt layer was separated, and successively washed with diluted hydrochloric acid, saturated sodium bicarbonate and water. After that, the AcOEt layer was dried with MgSO₄ overnight. The mixture was filtered and the filtrate was evaporated to dryness under vacuum, and the residue was recrystallized with ethyl acetate-cyclo hexane to yield 34.2 g target compound with the yield of 75%, m.p.=122~123° C., [α]_D²⁰=-1.8° (C=9.8 mg/mL, DMF)

¹H-NMR (300 MHz, DMSO-d₆): 1.36 (9H, s, -C(CH₃)₃), 6.82 (1H, d, J=8.4 Hz, 4-H), 3.86 (1H, m, 5-H), 7.01 (1H, s, 7-H_a), 7.31 (1H, s, 7-H_b), 1.73 (1H, m, 8-H_a), 1.88 (1H, m, 8-H_b), 2.36 (2H, t, J=7.2 Hz, 9-H), 5.07 (2H, s, 11-H), 7.25-7.39 (5H, m, 12~16-H).

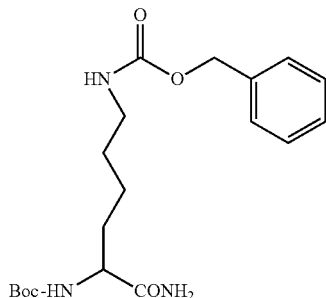
¹³C-NMR (125 MHz, DMSO-d₆): 28.1 (1-C), 78.0 (2-C), 155.3 (3-C), 53.3 (5-C), 173.5 (6-C), 27.1 (8-C), 30.2 (9-C), 172.2 (10-C), 65.4 (11-C), 127.8 (12 and 16-C), 128.4 (13 and 15-C), 127.9 (14-C).

ESI-MS: 337.75 [M+H]⁺, 673.32 [2M+H]⁺.

HR-MS(TOF): 337.1754 [M+H]⁺, 359.1572 [M+Na]⁺, C₁₇H₂₄N₂O₅.

Example 5

Liquid-phase synthesis of Boc-Lys(Z)-NH



To a solution of 38.0 g (1.0 eq) Boc-Lys(Z)-OH in tetrahydrofuran, 13.8 g (1.2 eq) HOSu and 18.9 ml (1.2 eq) DIC were

36

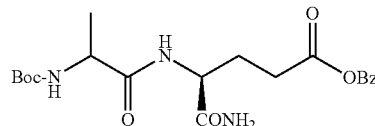
added, and the mixture was stirred in ice bath for 5 hours, and continued at r.t for 20 hours. A large number of white precipitate (DIU) was precipitated. The mixture was filtered, and the precipitate was washed with tetrahydrofuran. The filtrate was stirred in sodium chloride cryohydrate bath, and the anhydrous ammonia gas was introduced into the filtrate. 15 minutes later, a large number of white precipitate formed, and the reaction was continued for 1.5 hours, no more white precipitate formed, and the reaction was completed. The mixture was filtered, and the precipitate was washed with tetrahydrofuran. The filtrate was evaporated to dryness under vacuum and white solid residue was obtained. The residue was dissolved in AcOEt, the pH of the solution was adjusted to 7 with 2 N HCl aqueous solution in ice bath, and the solution was allowed to stand for 30 minutes. The AcOEt layer was separated, successively washed with diluted hydrochloric acid, saturated sodium bicarbonate aqueous solution and water, and dried with MgSO₄ overnight. The mixture was filtered, and the filtrate was evaporated to dryness under vacuum, the residue was recrystallized in AcOEt to obtain 35.0 g target compound with the yield of 92%, m.p.=137~138° C.

¹H-NMR (300 MHz, DMSO-d₆): 1.37 (9H, br.s, 1-H), 6.71 (1H, d, J=8.1 Hz, 4-H), 3.79 (1H, m, 5-H), 7.23 (2H, br.s, 7-H), 1.28 (2H, m, 8-H), 1.45 (2H, m, 9-H), 1.58 (2H, m, 10-H), 2.95 (2H, m, 11-H), 6.93 (1H, br.s, 12-H), 5.00 (2H, s, 14-H), 7.22-7.39 (5H, m, 16~20-H).

ESI-MS: 380.71 [M+H]⁺, 759.50 [2M+H]⁺.

HR-MS(TOF): 380.2201 [M+H]⁺, 781.4102 [2M+Na]⁺, C₁₉H₂₉N₃O₅.

Example 6

Liquid-phase synthesis of bipeptid fragment
Boc-Ala-D-Glu(OBzl)-NH₂

16.9 g (1.0 eq) Boc-Ala-OH was dissolved in tetrahydrofuran, 12.3 g (1.2 eq) HOSu and 16.9 mL (1.2 eq) DIC were added in sequence, the mixture was stirred in ice bath for 5 hours, and continued for 20 hours at r.t. A large amount of white precipitate (DIU) formed. The mixture was filtered, and the precipitate was washed with a small amount of tetrahydrofuran, and the filtrate containing (Boc-Ala-OSu) was collected for further use.

30 g (1.0 eq) Boc-D-Glu(OBzl)-NH₂ was dissolved in 100 mL trifluoroacetic acid-dichloromethane (v/v=1:1), and the solution was stirred for 1 hour at r.t to remove Boc group. After the completion of the reaction, the TFA was removed under vacuum; the residue was repeatedly grinded and washed in anhydrous ether, and evaporated to dryness, and re-dissolved in tetrahydrofuran. The pH of the solution was adjusted to 7~8 with N-methyl morpholine (NMM) in ice bath. The Boc-Ala-OSu solution was sparingly added to the solution in a few portions. The mixture was stirred for 5 hours in ice bath, and continued for 24 hours at r.t. After the completion of the reaction, the mixture was evaporated to dryness. The residue was dissolved in proper amount AcOEt and successively washed with diluted hydrochloric acid, saturated sodium bicarbonate aqueous solution and water. The AcOEt layer was separated, and dried with MgSO₄ overnight. The mixture was filtered and the filtrate was evaporated to dry-

37

ness. The residue was recrystallized from methanol and water, the crystal was washed with a big amount of ether to obtained 29.4 g target compound. Yield: 81%, m.p.=134~135° C.

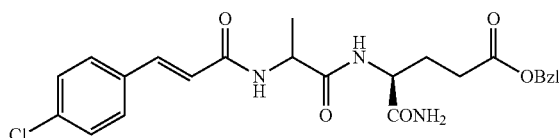
¹H-NMR (300 MHz, DMSO-d₆): 1.36 (9H, br.s, 1-H), 7.92 (1H, d, J=7.8 Hz, 4-H), 4.17 (1H, m, 5-H), 1.15 (3H, d, J=7.2 Hz, 6-H), 7.10 (1H, d, J=6.6 Hz, 8-H), 3.91 (1H, m, 9-H), 7.18 (1H, br.s, 11-H_a), 7.31 (1H, br.s, 11-H_b), 1.75 (1H, m, 12-H_a), 2.03 (1H, m, 12-H_b), 2.33 (2H, t, J=7.5 Hz, 13-H), 5.07 (2H, s, 15-H), 7.31-7.40 (5H, m, 17~21-H).

ESI-MS: 408.71 [M+H]⁺, 815.44 [2M+H]⁺.

HR-MS(TOF): 408.2137 [M+H]⁺, 430.1955 [M+Na]⁺, C₂₀H₂₉N₃O₆.

Example 7

Liquid-Phase Synthesis of Tripeptide Fragment



To a solution of 13.2 g (1.0 eq) 4-chloro cinnamic acid in tetrahydrofuran, 9.9 g (1.2 eq) HOSu and 13.6 mL (1.2 eq) DIC were added. The mixture was stirred for 5 hours in ice bath, continued for 20 hours at r.t. A large amount of white precipitate (DIU) formed. The mixture was filtered and the precipitate was washed with tetrahydrofuran; the filtrate (Ac-Osu) was collected for further use.

29.4 g (1.0 eq) Boc-Ala-D-Glu(OBzl)-NH₂ was dissolved in 100 mL trifluoroacetic acid-dichloromethane (v/v=1:1), and stirred for 1 hour to remove the Boc group. After completion of the reaction, TFA was removed under vacuum. The residue was repeatedly grinded, washed with ether, and evaporated to dryness and re-dissolved in tetrahydrofuran. The pH of the solution was adjusted to 7~8 with N-methyl morpholine (NMM) in ice bath. The Ac-Osu solution was sparingly added to the mixture in a few portions. The mixture was stirred for 5 hours in ice bath, then 24 hours at r.t, and refluxed for 2 hours. After completion of the reaction, the mixture was allowed to stand for 30 minutes and a large amount of viscous white precipitate formed. The mixture was filtered and the precipitate was washed with tetrahydrofuran. The precipitate was dissolved in AcOEt, and the solution was successfully washed with diluted hydrochloric acid, saturated sodium bicarbonate and water. The AcOEt layer was separated, and dried with MgSO₄ overnight. The mixture was filtered and the filtrate was evaporated to dryness. The residue was recrystallized in methanol-water, and washed with a

38

large amount of anhydrous ether to obtain 26.8 g target compound. Yield: 79%, m.p.=226~228° C.

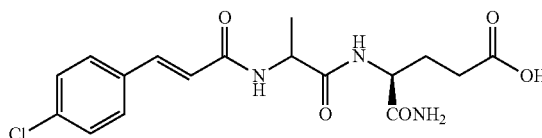
¹H-NMR (300 MHz, DMSO-d₆): 7.48 (2H, d, J=8.7 Hz, 2~6-H), 7.59 (2H, d, J=8.7 Hz, 3~5-H), 7.39 (1H, d, J=15.9 Hz, 7-H), 6.76 (1H, d, J=15.9 Hz, 8-H), 8.39 (1H, d, J=6.6 Hz, 10-H), 4.38 (1H, m, 11-H), 1.23 (3H, d, J=6.9 Hz, 12-H), 8.25 (1H, d, J=8.1 Hz, 14-H), 4.18 (1H, m, 15-H), 7.16 (1H, br.s, 17-H_a), 7.31 (1H, br.s, 17-H_b), 1.78 (1H, m, 18-H_a), 2.05 (1H, m, 18-H_b), 2.38 (2H, m, 19-H), 5.07 (2H, s, 21-H), 7.31-7.36 (5H, m, 23~27-H).

ESI-MS: 472.33 [M+H]⁺, 943.17 [2M+H]⁺.

HR-MS(TOF): 472.1635 [M+H]⁺, 943.3174 [2M+H]⁺, C₂₄H₂₆ClN₃O₅.

Example 8

Liquid-Phase Synthesis of Tripeptide Fragment



26.8 g tripeptide fragment of example 7 was dissolved in hydrobromic acid/acetic acid solution. The solution was stirred for 2 hours to remove the protective group. After completion of the reaction, the solution was poured to ice water, and adjusted the pH of the mixture to 10~11 with 10% NaOH aqueous solution. After extracting with AcOEt, the pH of the solution was adjusted to 2~3 with 10% HCl aqueous solution. The water phase was extracted with AcOEt 3 times, and the organic layers were combined, washed with brine and dried over Na₂SO₄. The mixture was filtered and the filtrate was concentrated to a small amount of solution under vacuum. Adding ether, a large amount of white solid precipitated. The mixture was filtered, and the precipitate was dried to obtain 18.5 g target compound. Yield, 85%.

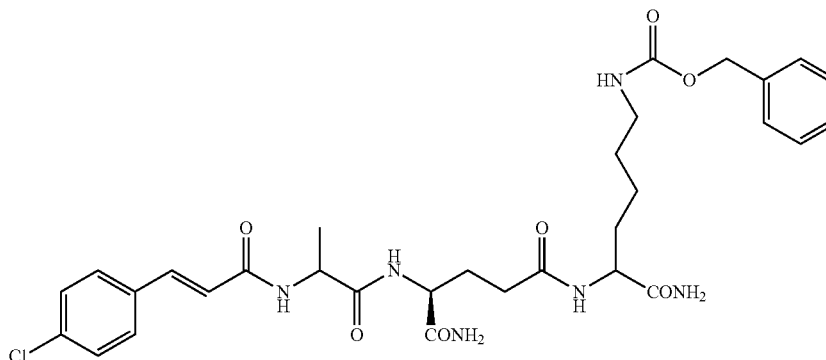
¹H-NMR (300 MHz, DMSO-d₆): 7.45 (2H, d, J=8.1 Hz, 2~6-H), 7.56 (2H, d, J=8.1 Hz, 3~5-H), 7.42 (1H, d, J=15.3 Hz, 7-H), 6.75 (1H, d, J=15.3 Hz, 8-H), 8.39 (1H, d, J=6.6 Hz, 10-H), 4.37 (1H, m, 11-H), 1.25 (3H, d, J=6.6 Hz, 12-H), 8.21 (1H, d, J=8.1 Hz, 14-H), 4.16 (1H, m, 15-H), 7.11 (1H, br.s, 17-H_a), 7.30 (1H, br.s, 17-H_b), 1.72 (1H, m, 18-H_a), 1.98 (1H, m, 18-H_b), 2.22 (2H, m, 19-H), 12.25 (1H, br.s, 21-H).

ESI-MS: 382.17 [M+H]⁺, 785.04 [2M+Na]⁺.

HR-MS(TOF): 382.1171 [M+H]⁺, 785.2073 [2M+Na]⁺, C₁₇H₂₀ClN₃O₅.

Example 9

Liquid-Phase Synthesis of Tetrapeptide Fragment



39

16.3 g (1.0 eq) tripeptide fragment of example 8 was dissolved in tetrahydrofuran, 5.9 g (1.2 eq) HOSu and 8.1 mL (1.2 eq) DIC were added in sequence. The mixture was stirred for 5 hours in ice bath, continued for 20 hours at r.t. A large amount of white solid (DIU) precipitated. The mixture was filtered and the precipitate was washed with a small amount of tetrahydrofuran, and the filtrate was collected for further use.

16.2 g (1.0 eq) Boc-Lys(Z)-NH₂ was dissolved in 100 mL trifluoroacetic acid-dichloromethane (v/v=1:1), and stirred for 1 hour to remove the Boc group. After completion of the reaction, the TFA was removed under vacuum, and the residue was repeatedly grinded and washed with ether, and evaporated to dryness. The residue was re-dissolved in tetrahydrofuran, and the pH was adjusted to 7~8 with N-methyl morpholine (NMM) in ice bath. The filtrate above was sparingly added to the solution in a few portions, and stirred in ice bath for 5 hours, and the reaction continued for 20 hours at r.t. A large amount of viscous white precipitate formed. The mixture was filtered and the precipitate was washed with a small amount of tetrahydrofuran. Then the precipitate was dried under vacuum, and 14.6 g target compounds was obtained with the yield of 74%, m.p.=195~196° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.47 (2H, m, 2 and 6-H), 7.58 (2H, m, 3 and 5-H), 7.38 (1H, d, J=15.3 Hz, 7-H), 6.79 (1H, d, J=15.3 Hz, 8-H), 8.45 (1H, d, J=8.1 Hz, 10-H), 4.40 (1H, m, 11-H), 1.28 (3H, m, 12-H), 8.29 (1H, d, J=8.1 Hz, 14-H), 4.19 (1H, m, 15-H), 6.95 (1H, s, 17_a-H), 7.41 (1H, s, 17_b-H), 1.71 (1H, m, 18-H), 1.96 (1H, m, 18_b-H), 2.14 (2H, m, 19-H), 7.92 (1H, m, 21-H), 4.12 (1H, m, 22-H), 7.09 (1H, s, 24_a-H), 7.33 (1H, m, 24_b-H), 1.49 (1H, m, 25_a-H), 1.65 (1H, m, 25_b-H), 1.27 (2H, m, 26-H), 1.53 (2H, m, 27-H), 2.91 (2H, m, 28-H), 6.91 (1H, br.s, 29-H), 5.00 (2H, s, 31-H), 7.20-7.38 (5H, m, 33~37-H).

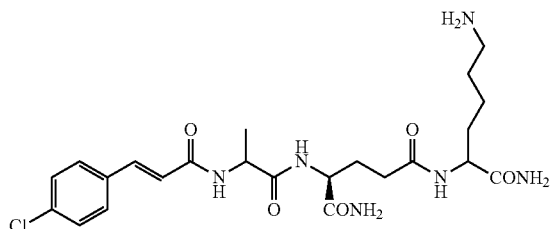
¹³C-NMR (125 MHz, DMSO-d₆): 133.9 (1-C), 129.0 (2 and 6-C), 129.2 (3 and 5-C), 133.8 (4-C), 137.6 (7-C), 122.8 (8-C), 164.7 (9-C), 48.9 (11-C), 18.1 (12-C), 172.4 (13-C), 52.1 (15-C), 173.9 (16-C), 27.6 (18-C), 31.6 (19-C), 171.5 (20-C), 52.1 (22-C), 173.3 (23-C), 31.4 (25-C), 22.7 (26-C), 27.5 (27-C), 38.7 (28-C), 156.0 (30-C), 65.1 (31-C), 137.5 (32-C), 127.7 (33 and 37-C), 128.3 (34 and 36-C), 127.0 (35-C).

ESI-MS: 643.31 [M+H]⁺.

HR-MS(TOF): 643.2635 [M+H]⁺, 665.2451 [M+Na]⁺, C₃₁H₃₉ClN₆O₇.

Example 10

Liquid-phase synthesis of Muramyl dipeptide Analogue MDA



14.6 g tripeptide fragment of example 9 was dissolved in a mixture of boron trifluoride diethyl etherate, trifluoroacetic acid and ethanol (v:v:v=9:9:2). The mixture was stirred at r.t. for 2 hours. After completion of the reaction, the solvent was evaporated to dryness under vacuum. Large amount of ether

40

was added to the residue in the ice bath, and white solid precipitated. The mixture was centrifuged, and the supernatant was separated. The precipitate was grinded and washed with large amount of ether repeatedly, and 8.3 g crude product was obtained with yield of 72%. The 8.3 g crude product was purified by ODS column chromatography with gradient elution method (methanol-water). The eluent was combined, and the solvent was removed under vacuum, and further dried by lypophilization, 6.8 g target compound was obtained with a purity of 98.5%. m.p.=215~217° C., [α]_D²⁰=+37.7° (C=11.05 mg/mL, DMF).

¹H-NMR (600 MHz, DMSO-d₆): 7.47 (2H, d, J=8.4 Hz, 2 and 6-H), 7.57 (2H, d, J=8.4 Hz, 3 and 5-H), 7.39 (1H, d, J=15.9 Hz, 7-H), 6.75 (1H, d, J=15.9 Hz, 8-H), 8.39 (1H, d, J=6.6 Hz, 10-H), 4.38 (1H, m, 11-H), 1.26 (3H, m, 12-H), 8.21 (1H, d, J=8.4 Hz, 14-H), 4.14 (1H, m, 15-H), 6.98 (1H, s, 17-H_a), 7.41 (1H, s, 17-H_b), 1.71 (1H, m, 18-H_a), 1.97 (1H, m, 18-H_b), 2.15 (2H, t, J=7.2 Hz, 19-H), 7.90 (1H, d, J=8.4 Hz, 21-H), 4.11 (1H, m, 22-H), 7.10 (1H, s, 24-H_a), 7.30 (1H, s, 24-H_b), 1.46 (1H, m, 25-H_a), 1.63 (1H, m, 25-H_b), 1.27 (2H, m, 26-H), 1.53 (2H, m, 27-H), 2.73 (2H, m, 28-H), 7.75 (2H, br.s, 29-H).

¹³C-NMR (150 MHz, DMSO-d₆): 134.0 (1-C), 129.0 (2 and 6-C), 129.2 (3 and 5-C), 133.8 (4-C), 137.6 (7-C), 122.7 (8-C), 164.7 (9-C), 48.8 (11-C), 18.1 (12-C), 172.4 (13-C), 52.2 (15-C), 173.8 (16-C), 27.7 (18-C), 31.7 (19-C), 171.6 (20-C), 52.1 (22-C), 173.3 (23-C), 31.3 (25-C), 22.4 (26-C), 26.8 (27-C), 38.7 (28-C).

IR: 3282.3, 3202.2 (ν_{OH} and ν_{NH}), 3067.3 (ν_{CH}), 2938.0 (ν_{CH}), 1609.5 (ν_{C=O}), 1537.5, 1450.2 (ν_{C=C}), 1199.0, 1180.2, 1130.6 (δ_{CH}), 972.4, 820.4, 799.4, 720.0 (δ_{CH} and ν_{C-Cl}).

ESI-MS: 509.60 [M+H]⁺, 1017.24 [2M+H]⁺.

HR-MS(TOF): 509.2292 [M+H]⁺, C₂₃H₃₃ClN₆O₅.

Example 11-22

Solid-Phase Synthesis of Muramyl Dipeptide Analogue

Example 11

Solid-Phase Synthesis of Muramyl Dipeptide MDA-201

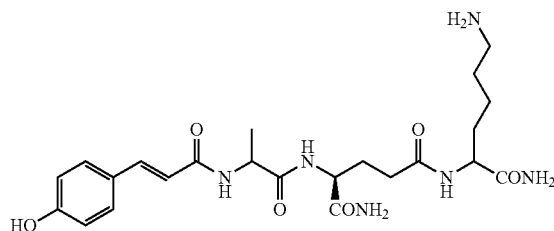
45

50

55

60

65



Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and p-hydroxycinnamic acid was introduced to the resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath and white solid precipitated. The mixture was

41

filtered, and the crude product was obtained, yield 85%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=143~144° C.

¹H-NMR (300 MHz, DMSO-d₆): 9.94 (1H, s, 1-OH), 6.79 (2H, d, J=8.7 Hz, 2 and 6-H), 7.59 (2H, d, J=8.7 Hz, 3 and 5-H), 7.36 (1H, d, J=15.9 Hz, 7-H), 6.51 (1H, d, J=15.9 Hz, 8-H), 8.25 (1H, d, J=6.3 Hz, 10-H), 4.34 (1H, m, 11-H), 1.24 (3H, m, 12-H), 8.17 (1H, d, J=8.4 Hz, 14-H), 4.12 (1H, m, 15-H), 6.98 (1H, s, 17-H_a), 7.31 (1H, s, 17-H_b), 1.72 (1H, m, 18-H_a), 1.98 (1H, m, 18-H_b), 2.15 (2H, m, 19-H), 7.89 (1H, d, J=7.8 Hz, 21-H), 4.11 (1H, m, 22-H), 7.10 (1H, s, 24-H_a), 7.31 (1H, s, 24-H_b), 1.48 (1H, m, 25-H_a), 1.63 (1H, m, 25-H_b), 1.25 (2H, m, 26-H), 1.50 (2H, m, 27-H), 2.74 (2H, m, 28-H), 7.76 (2H, br.s, 29-H).

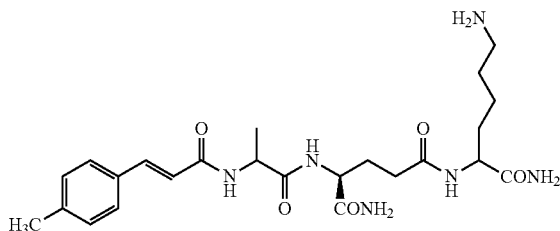
¹³C-NMR (125 MHz, DMSO-d₆): 159.0 (1-C), 115.8 (2 and 6-C), 129.3 (3 and 5-C), 125.8 (4-C), 139.2 (7-C), 118.2 (8-C), 165.5 (9-C), 48.9 (11-C), 17.9 (12-C), 172.6 (13-C), 52.2 (15-C), 173.8 (16-C), 27.6 (18-C), 31.7 (19-C), 171.6 (20-C), 52.1 (22-C), 173.3 (23-C), 31.3 (25-C), 22.4 (26-C), 26.7 (27-C), 38.7 (28-C).

IR: 3273.8, 3194.6 (ν_{OH} and ν_{NH}), 3064.6 (ν_{CH}), 2943.4 (ν_{CH}), 1663.6 (ν_{C=O}), 1605.7, 1537.3, 1515.0, 1450.4 (ν_{C=C}), 1201.6, 1180.2, 1135.7 (δ_{CH}), 983.8, 835.0, 800.4, 721.6 (δ_{CH}).

ESI-MS: 491.39 [M+H]⁺, 981.21 [2M+H]⁺.

HR-MS(TOF): 491.2597 [M+H]⁺, C₂₃H₃₄N₆O₆.

Example 12

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-202

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 4-methylcinnamic acid were introduced to resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was moved to an ice bath, and a large amount of ether was added to the residue, white solid precipitated immediately. The mixture was filtered, and the crude product was obtained, yield 86%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=150~151° C.

¹H-NMR (300 MHz, DMSO-d₆): 2.30 (3H, s, 1-CH₃), 7.44 (2H, d, J=8.1 Hz, 2 and 6-H), 7.21 (2H, d, J=8.1 Hz, 3 and 5-H), 7.37 (1H, d, J=15.9 Hz, 7-H), 6.69 (1H, d, J=15.9 Hz, 8-H), 8.35 (1H, d, J=6.6 Hz, 10-H), 4.37 (1H, m, 11-H), 1.25 (3H, m, 12-H), 8.21 (1H, d, J=8.1 Hz, 14-H), 4.12 (1H, m, 15-H), 6.99 (1H, s, 17-H_a), 7.32 (1H, s, 17-H_b), 1.73 (1H, m, 18-H_a), 1.97 (1H, m, 18-H_b), 2.16 (2H, m, 19-H), 7.90 (1H, d,

42

J=7.8 Hz, 21-H), 4.10 (1H, m, 22-H), 7.11 (1H, s, 24-H_a), 7.34 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.63 (1H, m, 25-H_b), 1.28 (2H, m, 26-H), 1.51 (2H, m, 27-H), 2.74 (2H, m, 28-H), 7.80 (2H, br.s, 29-H).

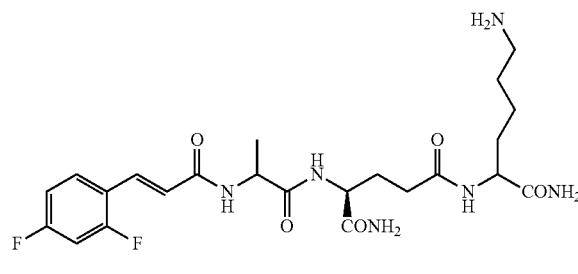
¹³C-NMR (125 MHz, DMSO-d₆): 20.9 (1-CH₃), 139.0 (2 and 6-C), 129.6 (2 and 6-C), 127.5 (3 and 5-C), 132.1 (4-C), 139.3 (7-C), 120.8 (8-C), 165.2 (9-C), 48.9 (11-C), 18.0 (12-C), 172.5 (13-C), 52.2 (15-C), 173.9 (16-C), 27.6 (18-C), 31.8 (19-C), 171.7 (20-C), 52.1 (22-C), 173.4 (23-C), 31.3 (25-C), 22.4 (26-C), 26.7 (27-C), 38.7 (28-C).

IR: 3278.8, 3199.9 (ν_{OH} and ν_{NH}), 3063.3 (ν_{CH}), 2941.3 (ν_{CH}), 1656.3 (ν_{C=O}), 1540.7, 1452.5 (ν_{C=C}), 1202.2, 1184.1, 1135.3 (δ_{CH}), 984.0, 835.8, 813.6, 800.7, 721.6 (δ_{CH}).

ESI-MS: 489.48 [M+H]⁺, 977.29 [2M+H]⁺.

HR-MS(TOF): 489.2819 [M+H]⁺, C₂₄H₃₆N₆O₅.

Example 13

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-203

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2,4-difluorocinnamic acid was introduced to resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was moved to an ice bath, and a large amount of ether was added to the residue, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 80%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=189~190° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.35 (1H, m, 2-H), 7.72 (1H, dd, J=15.2 and 8.7 Hz, 5-H), 7.18 (1H, td, J=8.4 and 2.4 Hz, 6-H), 7.44 (1H, d, J=15.9 Hz, 7-H), 6.82 (1H, d, J=15.9 Hz, 8-H), 8.51 (1H, d, J=6.6 Hz, 10-H), 4.40 (1H, m, 11-H), 1.27 (3H, d, J=7.2 Hz, 12-H), 8.24 (1H, d, J=8.1 Hz, 14-H), 4.17 (1H, m, 15-H), 7.00 (1H, s, 17-H_a), 7.33 (1H, s, 17-H_b), 1.71 (1H, m, 18-H_a), 1.97 (1H, m, 18-H_b), 2.17 (2H, t, J=7.8 Hz, 19-H), 7.91 (1H, d, J=8.4 Hz, 21-H), 4.13 (1H, m, 22-H), 7.07 (1H, s, 24-H_a), 7.32 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.64 (1H, m, 25-H_b), 1.29 (2H, m, 26-H), 1.50 (2H, m, 27-H), 2.75 (2H, m, 28-H).

¹³C-NMR (125 MHz, DMSO-d₆): 163.7 (m, 1-C), 104.7 (t, J=26.0 Hz, 2-C), 159.6 (m, 3-C), 118.5 (m, 4-C), 130.6 (m, 5-C), 112.4 (d, J=18.4 Hz, 6-C), 137.4 (s, 7-C), 124.3 (s, 8-C), 164.7 (s, 9-C), 48.9 (11-C), 18.0 (12-C), 172.2 (13-C), 52.1

43

(15-C), 173.2 (16-C), 27.6 (18-C), 31.7 (19-C), 171.6 (20-C), 52.0 (22-C), 172.3 (23-C), 31.3 (25-C), 22.4 (26-C), 26.8 (27-C), 38.7 (28-C).

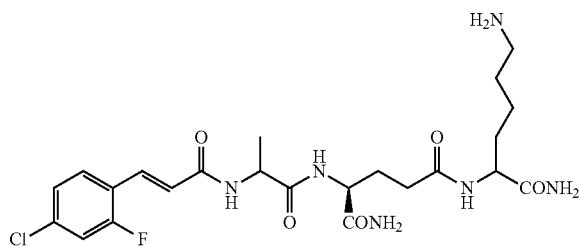
IR: 3279.8, 3198.2 (ν_{OH} and ν_{NH}), 3066.7 ($\nu_{=CH}$), 2939.5 (ν_{-CH}), 1656.2 ($\nu_{C=O}$), 1616.4, 1544.6, 1504.2, 1454.1 ($\nu_{C=C}$), 1202.1, 1181.7, 1138.8 (ν_{C-F} and δ_{-CH}), 967.5, 836.7, 800.7, 721.4 (ν_{C-Cl} and $\delta_{=CH}$).

ESI-MS: 511.28 [M+H]⁺, 1021.02 [2M+H]⁺.

HR-MS(TOF): 511.2482 [M+H]⁺, C₂₄H₃₆N₆O₅.

Example 14

Solid-Phase Synthesis of Muramyl Dipeptide MDA-204



Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 4-chloro-2-fluorocinnamic acid was introduced to the resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 88%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lyophilization. m.p.=149~150° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.54 (1H, dd, J=10.8 and 1.8 Hz, 2-H), 7.69 (1H, t, J=8.7 Hz, 5-H), 7.36 (1H, dd, J=10.5 and 2.1 Hz, 6-H), 7.44 (1H, d, J=15.9 Hz, 7-H), 6.87 (1H, d, J=15.9 Hz, 8-H), 8.57 (1H, d, J=6.6 Hz, 10-H), 4.40 (1H, m, 11-H), 1.27 (3H, d, J=7.2 Hz, 12-H), 8.27 (1H, d, J=8.1 Hz, 14-H), 4.13 (1H, m, 15-H), 6.99 (1H, s, 17-H_a), 7.35 (1H, s, 17-H_b), 1.72 (1H, m, 18-H_a), 1.98 (1H, m, 18-H_b), 2.17 (2H, t, J=7.8 Hz, 19-H), 8.08 (1H, d, J=8.1 Hz, 21-H), 4.10 (1H, m, 22-H), 7.12 (1H, s, 24-H_a), 7.32 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.64 (1H, m, 25-H_b), 1.29 (2H, m, 26-H), 1.51 (2H, m, 27-H), 2.74 (2H, m, 28-H).

¹³C-NMR (125 MHz, DMSO-d₆): 135.1 (d, J=10.9 Hz, 1-C), 117.2 (d, J=25.8 Hz, 2-C), 160.7 (d, J=252.5 Hz, 3-C), 122.1 (d, J=11.6 Hz, 4-C), 130.8 (s, 5-C), 125.9 (d, J=3.0 Hz, 6-C), 137.3 (m, 7-C), 125.8 (d, J=6.3 Hz, 8-C), 164.6 (s, 9-C), 49.4 (11-C), 18.5 (12-C), 172.8 (13-C), 52.7 (15-C), 174.3 (16-C), 28.1 (18-C), 32.2 (19-C), 172.1 (20-C), 52.6 (22-C), 173.8 (23-C), 31.8 (25-C), 22.9 (26-C), 27.5 (27-C), 38.7 (28-C).

IR: 3358.7, 3284.3, 3199.3 (ν_{OH} and ν_{NH}), 3067.3 ($\nu_{=CH}$), 2933.4 (ν_{-CH}), 1654.7, 1642.5, 1642.5, 1622.9 ($\nu_{C=O}$),

44

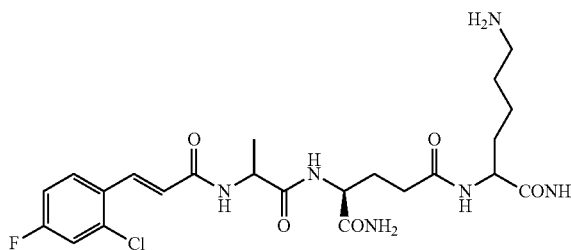
1540.6, 1489.9, 1453.6 ($\nu_{C=C}$), 1202.4, 1129.9 (ν_{C-F} and δ_{-CH}), 978.2, 815.0, 720.6, 690.2 (ν_{C-Cl} and $\delta_{=CH}$).

ESI-MS: 527.49 [M+H]⁺, 1053.17 [2M+H]⁺.

HR-MS(TOF): 527.2192 [M+H]⁺, C₂₃H₃₂ClFN₆O₅.

Example 15

Solid-Phase Synthesis of Muramyl Dipeptide MDA-205



Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2-chloro-4-fluorocinnamic acid were introduced to resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 86%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lyophilization. m.p.=137~138° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.55 (1H, dd, J=8.7 and 1.8 Hz, 2-H), 7.77 (1H, m, 5-H), 7.36 (1H, m, 6-H), 7.66 (1H, d, J=15.9 Hz, 7-H), 6.79 (1H, d, J=15.9 Hz, 8-H), 8.47 (1H, d, J=6.6 Hz, 10-H), 4.42 (1H, m, 11-H), 1.27 (3H, d, J=6.9 Hz, 12-H), 8.24 (1H, d, J=8.4 Hz, 14-H), 4.16 (1H, m, 15-H), 7.00 (1H, s, 17-H_a), 7.31 (1H, s, 17-H_b), 1.72 (1H, m, 18-H_a), 1.99 (1H, m, 18-H_b), 2.17 (2H, t, J=7.8 Hz, 19-H), 7.91 (1H, d, J=8.7 Hz, 21-H), 4.13 (1H, m, 22-H), 7.12 (1H, s, 24-H_a), 7.33 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.65 (1H, m, 25-H_b), 1.30 (2H, m, 26-H), 1.52 (2H, m, 27-H), 2.75 (2H, br.s, 28-H), 7.79 (2H, br.s, 29-H).

¹³C-NMR (125 MHz, DMSO-d₆): 162.7 (d, J=250.0 Hz, 1-C), 115.9 (d, J=21.6 Hz, 2-C), 134.6 (d, J=10.0 Hz, 3-C), 129.9 (d, J=3.8 Hz, 4-C), 129.7 (d, J=10.0 Hz, 5-C), 117.7 (d, J=25.1 Hz, 3-C), 137.5 (7-C), 125.4 (8-C), 164.8 (9-C), 49.3 (11-C), 18.6 (12-C), 172.1 (13-C), 52.6 (15-C), 174.2 (16-C), 28.2 (18-C), 32.2 (19-C), 172.1 (20-C), 52.5 (22-C), 173.7 (23-C), 31.8 (25-C), 22.9 (26-C), 27.2 (27-C), 38.2 (28-C).

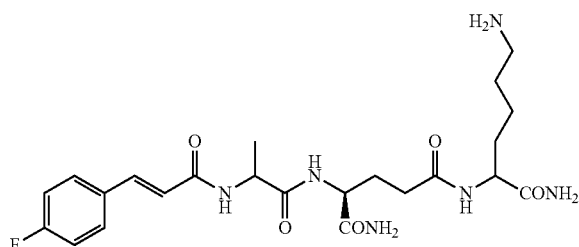
IR: 3279.8 (ν_{OH} and ν_{NH}), 3066.0 ($\nu_{=CH}$), 2937.1 (ν_{-CH}), 1776.1, 1656.3 ($\nu_{C=O}$), 1537.0, 1489.0, 1452.2 ($\nu_{C=C}$), 1238.1, 1201.1, 1181.0, 1135.6 (ν_{C-F} and δ_{-CH}), 910.6, 835.5, 800.1, 721.3 (ν_{C-Cl} and $\delta_{=CH}$).

ESI-MS: 527.28 [M+H]⁺, 1075.00 [2M+Na]⁺.

HR-MS(TOF): 527.2201 [M+H]⁺, C₂₃H₃₂ClFN₆O₅.

45

Example 16

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-206

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 4-fluorocinnamic acid were introduced in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, and the residue was subjected to a large amount of ether in ice bath, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained, yield 92%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=218~220° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.26 (2H, t, J=8.7 Hz, 2 and 6-H), 7.63 (2H, dd, J=8.4 and 5.7 Hz, 3 and 5-H), 7.42 (1H, d, J=15.9 Hz, 7-H), 6.71 (1H, d, J=15.9 Hz, 8-H), 8.37 (1H, d, J=6.6 Hz, 10-H), 4.40 (1H, m, 11-H), 1.27 (3H, d, J=7.2 Hz, 12-H), 8.21 (1H, d, J=8.1 Hz, 14-H), 4.15 (1H, m, 15-H), 7.00 (1H, s, 17-H_a), 7.32 (1H, s, 17-H_b), 1.71 (1H, m, 18-H_a), 1.99 (1H, m, 18-H_b), 2.17 (2H, t, J=7.8 Hz, 19-H), 7.90 (1H, d, J=8.1 Hz, 21-H), 4.14 (1H, m, 22-H), 7.12 (1H, s, 24-H_a), 7.32 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.64 (1H, m, 25-H_b), 1.29 (2H, m, 26-H), 1.52 (2H, m, 27-H), 2.76 (2H, m, 28-H), 7.71 (2H, br.s, 29-H).

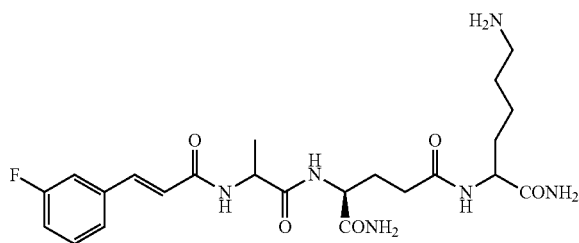
¹³C-NMR (125 MHz, DMSO-d₆): 163.2 (d, J=245.8 Hz, 1-C), 116.4 (d, J=21.6 Hz, 2 and 6-C), 130.1 (d, J=8.5 Hz, 3 and 5-C), 131.9 (4-C), 138.3 (7-C), 122.2 (8-C), 165.3 (9-C), 49.3 (11-C), 18.5 (12-C), 172.8 (13-C), 52.6 (15-C), 174.2 (16-C), 27.2 (18-C), 32.2 (19-C), 172.1 (20-C), 52.5 (22-C), 173.7 (23-C), 31.8 (25-C), 22.9 (26-C), 27.2 (27-C), 38.5 (28-C).

IR: 3278.5, 3198.1 (ν_{OH} and ν_{NH}), 3068.1 (ν_{CH}), 2931.9 (ν_{CH}), 1672.8, 1639.9 (ν_{C=O}), 1614.9, 1539.4, 1509.6, 1451.7 (ν_{C=C}), 1201.7, 1134.3 (ν_{C-F} and δ_{CH}), 971.4, 831.4, 800.6, 721.0 (δ_{CH}).

ESI-MS: 493.25 [M+H]⁺, 1007.02 [2M+Na]⁺.

HR-MS(TOF): 493.2580 [M+H]₄⁺, 515.2381 [M+Na]₄⁺, C₂₃H₃₃FN₆O₅.

Example 17

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-207

46

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 4-fluorocinnamic acid was introduced in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, and, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained, yield 75%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=195~196° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.21 (1H, s, 2-H), 7.38 (1H, m, 3-H), 7.41 (1H, m, 5-H), 7.47 (1H, m, 6-H), 7.47 (1H, d, J=15.9 Hz, 7-H), 6.79 (1H, d, J=15.9 Hz, 8-H), 8.39 (1H, d, J=6.0 Hz, 10-H), 4.38 (1H, m, 11-H), 1.26 (3H, d, J=6.9 Hz, 12-H), 8.22 (1H, d, J=7.5 Hz, 14-H), 4.13 (1H, m, 15-H), 6.97 (1H, s, 17-H_a), 7.30 (1H, s, 17-H_b), 1.65 (1H, m, 18-H_a), 1.97 (1H, a, 18-H_b), 2.15 (2H, m, 19-H), 7.90 (1H, d, J=8.4 Hz, 21-H), 4.13 (1H, m, 22-H), 7.01 (1H, s, 24-H_a), 7.30 (1H, s, 24-H_b), 1.48 (1H, m, 25-H_a), 1.65 (1H, m, 25-H_b), 1.28 (2H, m, 26-H), 1.48 (2H, m, 27-H), 2.72 (2H, m, 28-H).

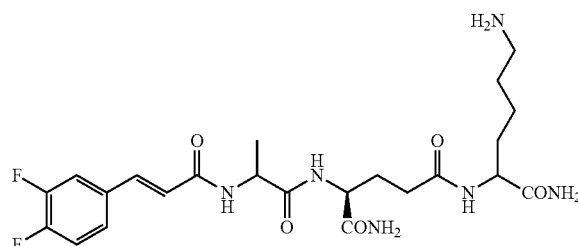
¹³C-NMR (125 MHz, DMSO-d₆): 116.7 (d, J=21.0 Hz, 1-C), 162.9 (d, J=242.3 Hz, 2-C), 114.4 (d, J=21.4 Hz, 3-C), 137.9 (d, J=7.8 Hz, 4-C), 124.0 (d, J=22.6 Hz, 5-C), 131.4 (6-C), 138.1 (7-C), 124.0 (8-C), 165.1 (9-C), 49.3 (11-C), 18.6 (12-C), 172.8 (13-C), 52.6 (15-C), 174.3 (16-C), 28.2 (18-C), 32.2 (19-C), 172.0 (20-C), 52.5 (22-C), 173.7 (23-C), 31.8 (25-C), 22.9 (26-C), 27.2 (27-C), 38.5 (28-C).

IR: 3276.4, 3201.1 (ν_{OH} and ν_{NH}), 3069.1 (ν_{CH}), 2938.1 (ν_{CH}), 1647.7 (ν_{C=O}), 1539.0, 1448.0, 1421.8 (ν_{C=C}), 1200.8, 1180.2, 1134.1 (ν_{C-F} and δ_{CH}), 972.1, 834.9, 798.7, 721.2 (δ_{CH}).

ESI-MS: 493.25 [M+H]⁺, 1007.09 [2M+Na]⁺.

HR-MS(TOF): 493.2582 [M+H]⁺, C₂₃H₃₃FN₆O₅.

Example 18

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-208

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 3,4-difluorocinnamic acid were introduced to the resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, and a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 95%. The crude product was purified by ODS column chromatography, and white solid with a purity of 98.5% was obtained through lypophilization. m.p.=139~140° C.

¹H-NMR (300 MHz, DMSO-d₆): 7.66 (1H, m, 3-H), 7.48 (1H, m, 5-H), 7.45 (1H, m, 6-H), 7.40 (1H, d, J=15.9 Hz, 7-H), 6.75 (1H, d, J=15.9 Hz, 8-H), 8.37 (1H, d, J=6.9 Hz,

47

10-H), 4.40 (1H, m, 11-H), 1.27 (3H, d, $J=7.2$ Hz, 12-H), 8.22 (1H, d, $J=7.8$ Hz, 14-H), 4.16 (1H, m, 15-H), 7.00 (1H, s, 17-H_a), 7.33 (1H, s, 17-H_b), 1.71 (1H, m, 18-H_a), 1.97 (1H, m, 18-H_b), 2.17 (2H, t, $J=7.8$ Hz, 19-H), 7.90 (1H, d, $J=8.1$ Hz, 21-H), 4.13 (1H, m, 22-H), 7.12 (1H, s, 24-H_a), 7.31 (1H, s, 24-H_b), 1.49 (1H, m, 25-H_a), 1.65 (1H, m, 25-H_b), 1.29 (2H, m, 26-H), 1.52 (2H, m, 27-H), 2.76 (2H, m, 28-H), 7.73 (2H, br.s, 29-H).

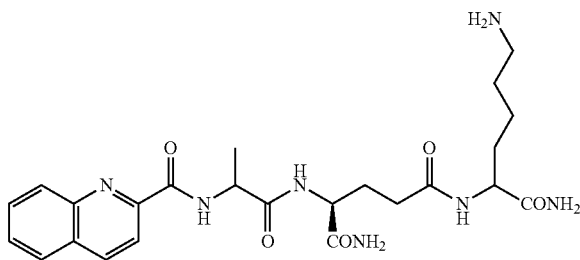
¹³C-NMR (150 MHz, DMSO-d₆): 149.3 (dd, $J=35.6$ and 12.8 Hz, 1-C), 151.2 (dd, $J=38.5$ and 12.9 Hz, 2-C), 118.6 (d, $J=17.5$ Hz, 3-C), 133.3 (m, 4-C), 125.1 (m, 5-C), 116.7 (d, $J=17.4$ Hz, 6-C), 137.3 (s, 7-C), 123.8 (s, 8-C), 165.0 (9-C), 49.3 (11-C), 18.6 (12-C), 172.8 (13-C), 52.6 (15-C), 174.3 (16-C), 28.2 (18-C), 31.8 (19-C), 172.1 (20-C), 52.5 (22-C), 173.7 (23-C), 31.8 (25-C), 22.9 (26-C), 27.2 (27-C), 38.2 (28-C).

IR: 3275.8, 3196.4 (ν_{OH} and ν_{NH}), 3064.8 (ν_{CH}), 2938.1 (ν_{CH}), 1673.1 ($\nu_{C=O}$), 1612.9, 1542.1, 1516.7, 1451.5 ($\nu_{C=C}$), 1201.6, 1135.4 (ν_{C-F} and δ_{CH}), 969.3, 834.3, 800.6, 721.2 (δ_{CH}).

ESI-MS: 511.30 [M+H]⁺, 1021.09 [2M+H]⁺.

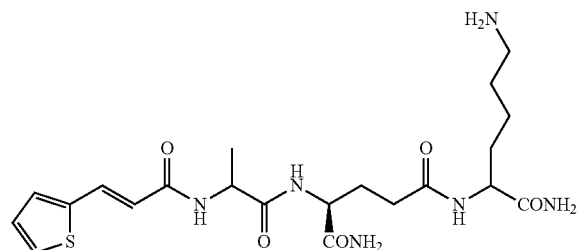
HR-MS(TOF): 511.2479 [M+H]⁺, C₂₃H₃₂F₂N₆O₅.

Example 19

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-113

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2-quinolinecarboxylic acid were introduced to resin in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained, yield 80%. The crude product was purified by ODS column chromatography, and MDA-113 as white solid with a purity of 98.5% was obtained through lypophilization.

Example 20

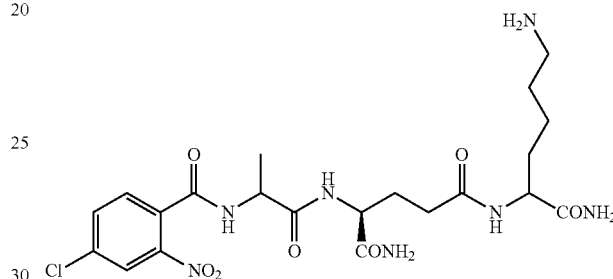
Solid-Phase Synthesis of Muramyl Dipeptide
MDA-119

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-

48

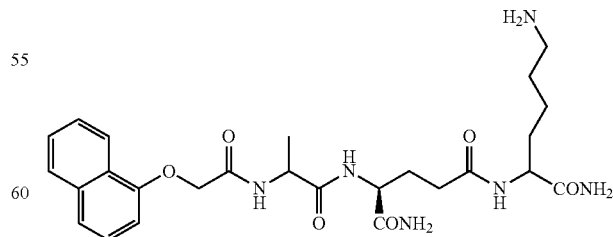
Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2-thienylacrylic acid were introduced in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 83%. The crude product was purified by ODS column chromatography, and MDA-119 as white solid with a purity of 98.5% was obtained through lypophilization.

Example 21

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-130

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2-thienylacrylic acid were introduced in sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent was drained, and the resin was cleaved for 1 hour in 90% (volume percentage) TFA aqueous solution. The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, and, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 81%. The crude product was purified by ODS column chromatography, and MDA-130 as white solid with a purity of 98.5% was obtained through lypophilization.

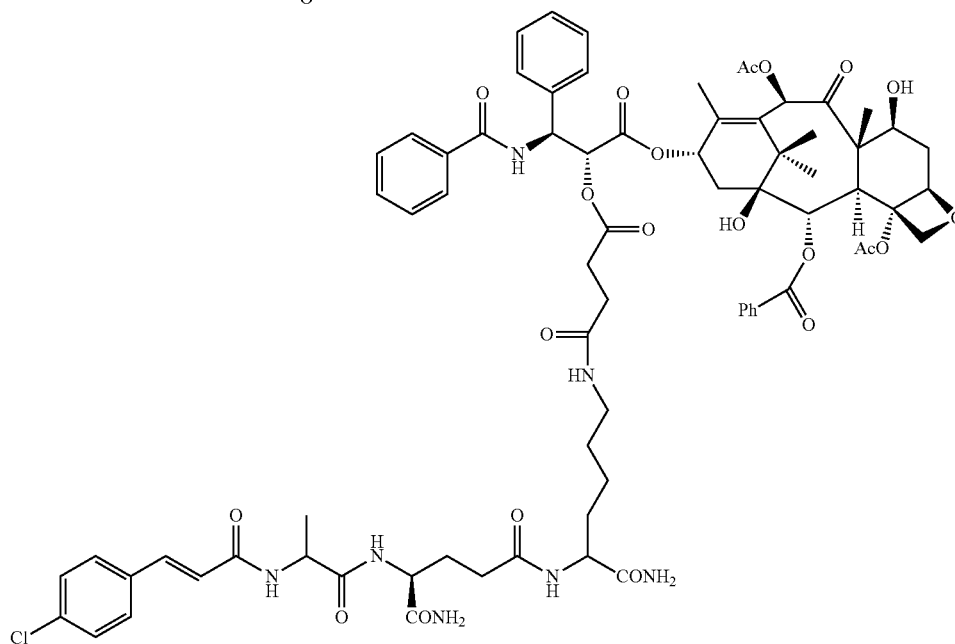
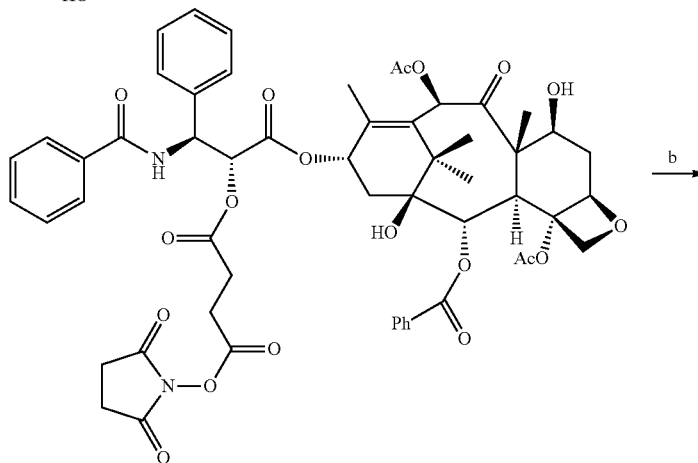
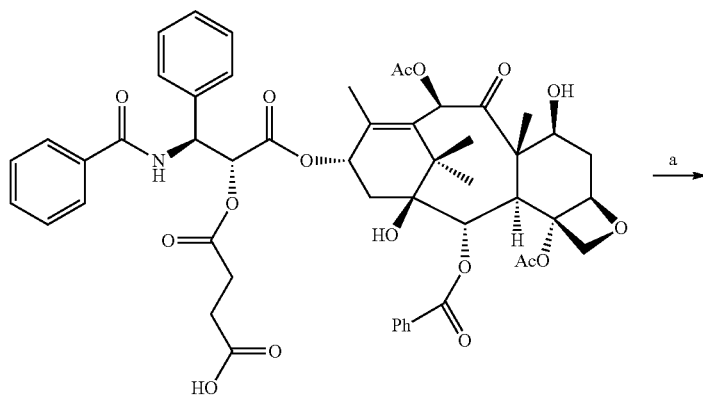
Example 22

Solid-Phase Synthesis of Muramyl Dipeptide
MDA-133

Solid-phase synthesis strategy was employed. Rink-Amide AM resin (loading 0.88 mmol/g) was chosen, Fmoc-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-Ala-COOH and 2-naphthoxyacetic acid were introduced in

49

sequence. After the completion of the condensation, the resin was sufficiently washed and the solvent drained, and the resin was cleaved for 1 hour in 90% trifluoroacetic acid aqueous solution (Volume percentage). The solvent was removed under vacuum, the residue was subjected to a large amount of ether in ice bath, and, a white solid precipitated immediately. The mixture was filtered, and the crude product was obtained with yield of 88%. The crude product was purified by ODS column chromatography, and MDA-133 as white solid with a purity of 98.5% was obtained through lypophilization.



50

Example 23-35

Liquid-Phase Synthesis of MTC Conjugates

Example 23

Liquid-Phase Synthesis of Conjugate MTC-220

The synthetic route was shown below:

51

Reagents and conditions: (a) HOSu, EDC.HCl, DMSO, r.t, 20 h; (b) MDA, DMSO, r.t, 12 h.

9.53 g (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 1.15 g (1.0 eq) HOSu and 1.92 g (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 20 hours. 5.08 g (1.0 eq) muramyl dipeptide analogue (MDA) was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 20 hours. After the completion of the reaction, plenty of water was added to the mixture, and a white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 11.8 g solid product was obtained through lypophilization. Yield 82%, m.p.=180~181° C., $[\alpha]_D^{25} = -9.8^\circ$ (C=10.1 mg/mL, DMF).

$^1\text{H-NMR}$ (600 MHz, DMSO- d_6): 4.63 (1H, br.s, 1-OH), 5.42 (1H, d, J=7.2 Hz, 2-H), 3.58 (1H, d, J=7.2 Hz, 3-H), 4.90 (1H, m, 5-H), 1.62 (1H, m, 6- H_a), 2.30 (1H, m, 6- H_b), 4.12 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.30 (1H, s, 10-H), 5.82 (1H, t, J=9.0 Hz, 13-H), 1.46 (1H, m, 14- H_a), 1.79 (1H, m, 14- H_b), 1.00 (3H, s, 16-H), 1.03 (3H, s, 17-H), 1.77 (3H, s, 18-H), 1.50 (3H, s, 19-H), 3.99 (1H, d, J=9.0 Hz, 20- H_a), 4.02 (1H, d, J=9.0 Hz, 20- H_b), 2.24 (3H, s, 4-OCOCH₃), 2.11 (3H, s, 10-OCOCH₃), 5.34 (1H, d, J=9.0 Hz, 2'-H), 5.54 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=9.0 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.46 (2H, m, ph-m-H), 7.55 (1H, t, J=7.2 Hz, ph-p-H), 7.83 (2H, m, NBz-o-H), 7.44 (2H, m, NBz-m-H), 7.19 (1H, m, NBz-p-H), 7.98 (2H, d, J=7.2 Hz, OBz-o-H), 7.66

52

J=15.6 Hz, 46-H), 7.41 (1H, d, J=15.6 Hz, 47-H), 7.58 (2H, d, J=9.0 Hz, 49 and 53-H), 7.49 (2H, d, J=9.0 Hz, 50 and 52-H).

$^{13}\text{C-NMR}$ (150 MHz, DMSO- d_6): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.2 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.8, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.4 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.3 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.5 (27-C), 22.9 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.3 (41-C), 48.8 (42-C), 18.1 (43-C), 164.7 (45-C), 122.7 (46-C), 137.6 (47-C), 133.8 (48-C), 129.0 (49 and 53-C), 129.2 (50 and 52-C), 133.9 (51-C).

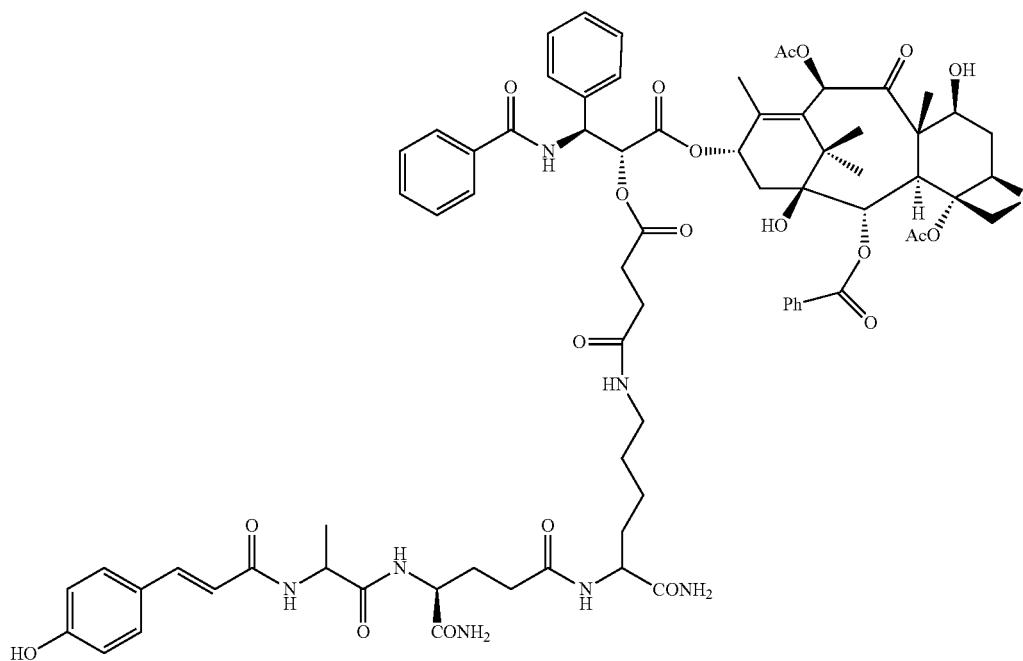
IR: 3316.9 (ν_{OH} and ν_{NH}), 3066.0 (ν_{CH}), 2935.0, 2873.1 (ν_{CH}), 1736.0, 1655.0 ($\nu_{\text{C=O}}$), 1537.3, 1492.9 ($\nu_{\text{C=C}}$), 1451.7, 1371.8 (δ_{CH}), 1241.5 ($\nu_{\text{C-O-C}}$), 980.2, 906.6, 822.6, 776.2, 708.9 ($\delta_{\text{=CH}}$).

ESI-MS: 1444.56 [M+H]⁺, 1466.46 [M+Na]⁺.

HR-MS(TOF): 1444.5645 [M+H]⁺, 1466.5475 [M+Na]⁺, C₇₄H₈₆ClN₇O₂₁.

Example 24

Liquid-Phase Synthesis of Conjugate MTC-301



(2H, t, J=7.2 Hz, OBz-m-H), 7.74 (1H, t, J=7.2 Hz, OBz-p-H), 2.61 (2H, m, 22-H), 2.36 (2H, t, J=7.2 Hz, 23-H), 7.82 (1H, m, 25-H), 2.90 (1H, m, 26- H_a), 3.00 (1H, m, 26- H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45 (1H, m, 29- H_a), 1.63 (1H, m, 29- H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32- H_a), 7.30 (1H, s, 32- H_b), 7.87 (1H, m, 33-H), 2.16 (2H, t, J=7.2 Hz, 35-H), 1.71 (1H, m, 36- H_a), 1.99 (1H, m, 36- H_b), 4.13 (1H, m, 37-H), 7.10 (1H, s, 39- H_a), 7.30 (1H, s, 39- H_b), 8.21 (1H, d, J=8.4 Hz, 40-H), 4.40 (1H, t, J=7.2 Hz, 42-H), 1.28 (3H, d, J=6.6 Hz, 43-H), 8.37 (1H, d, J=7.2 Hz, 44-H), 6.76 (1H, d,

953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 490 mg (1.0 eq) muramyl dipeptide analogue MDA-201 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS col-

53

umn chromatography, 11.8 g solid product was obtained through lypophilization. Yield, 83%, m.p.=179~180° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.62 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.89 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=7.5 Hz, 13-H), 1.46 (1H, m, 14-H_a), 1.75 (1H, m, 14-H_b), 1.01 (3H, s, 16-H), 1.04 (3H, s, 17-H), 1.78 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.99 (1H, d, J=8.5 Hz, 20-H_a), 4.00 (1H, d, J=8.5 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.10 (3H, s, 10-OCOCH₃), 5.33 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, d, J=7.5 Hz, ph-o-H), 7.47 (2H, d, J=7.5 Hz, ph-m-H), 7.55 (1H, t, J=7.5 Hz, ph-p-H), 7.83 (2H, m, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.17 (1H, m, NBz-p-H), 7.98 (2H, d, J=7.5 Hz, OBz-o-H), 7.65 (2H, t, J=8.0 Hz, OBz-m-H), 7.74 (1H, t, J=7.5 Hz, OBz-p-H), 2.72 (2H, m, 22-H), 2.35 (2H, t, J=7.0 Hz, 23-H), 7.82 (1H, m, 25-H), 2.96 (1H, m, 26-H_a), 3.00 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45

54

(OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.5 (27-C), 22.9 (28-C), 31.6 (29-C), 52.4 (30-C), 173.9 (31-C), 171.6 (34-C), 31.8 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.3 (41-C), 48.8 (42-C), 18.0 (43-C), 164.7 (45-C), 118.2 (46-C), 137.4 (47-C), 125.8 (48-C), 127.5 (49 and 53-C), 115.8 (50 and 52-C), 158.9 (51-C).

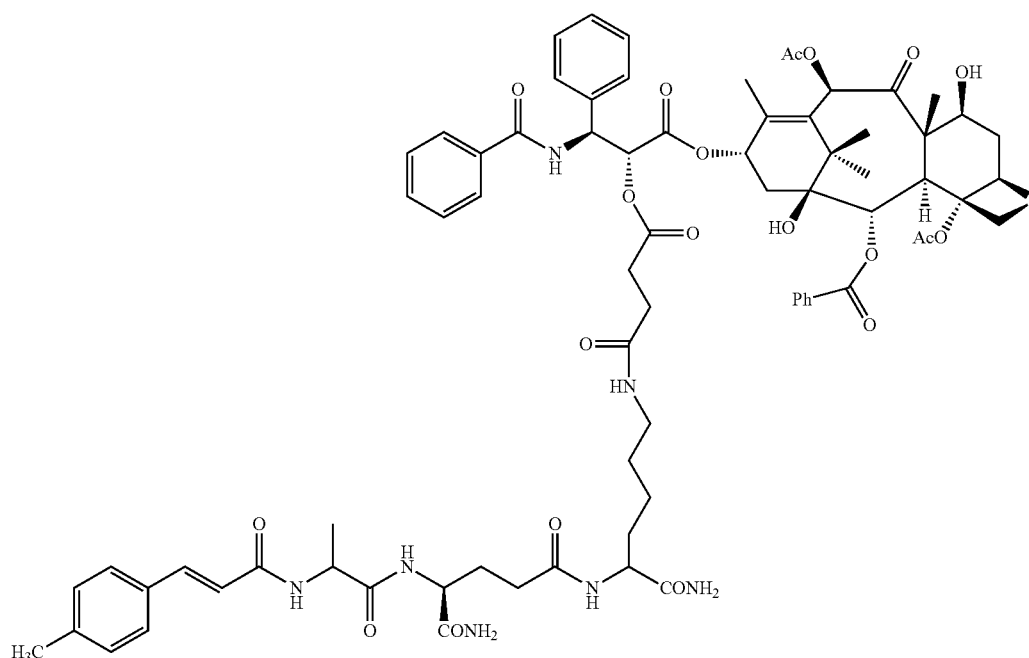
IR: 3324.4 (ν_{OH} and ν_{NH}), 3075.1 (ν_{CH}), 1740.6, 1657.2 (ν_{C=O}), 1603.9, 1518.3, 1450.8 (ν_{C=C}), 1243.4 (ν_{C-O-C}), 980.6, 710.3 (δ_{CH}).

ESI-MS: 1426.31 [M+H]⁺, 1449.03 [M+Na+H]²⁺.

HR-MS(TOF): 1426.5974 [M+H]⁺, 1448.5786 [M+Na]⁺, C₇₄H₈₇N₇O₂₂.

Example 25

Liquid-Phase Synthesis of Conjugate MTC-302



(1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.10 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, m, 32-H_b), 7.86 (1H, m, 33-H), 2.14 (2H, t, J=8.0 Hz, 35-H), 1.75 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.11 (1H, m, 37-H), 7.10 (1H, s, 39-H_a), 7.30 (1H, m, 39-H_b), 8.19 (1H, d, J=8.0 Hz, 40-H), 4.36 (1H, m, 42-H), 1.25 (3H, d, J=7.0 Hz, 43-H), 8.22 (1H, d, J=6.5 Hz, 44-H), 6.51 (1H, d, J=15.5 Hz, 46-H), 7.32 (1H, d, J=15.5 Hz, 47-H), 7.46 (2H, d, J=8.5 Hz, 49 and 53-H), 6.78 (2H, d, J=8.5 Hz, 50 and 52-H), 9.85 (1H, s, 51-OH).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.3 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.2 (12-C), 70.4 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.6, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.2 (1'-C), 74.4 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6

953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 488 mg (1.0 eq) muramyl dipeptide analogue MDA-202 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.09 g solid product was obtained through lypophilization. Yield, 77%, m.p.=172~174° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.63 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.89 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=9.5 Hz, 13-H), 1.46 (1H, m, 14-H_a), 1.79 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.99 (1H, d, J=8.0 Hz, 20-H_a), 4.01

55

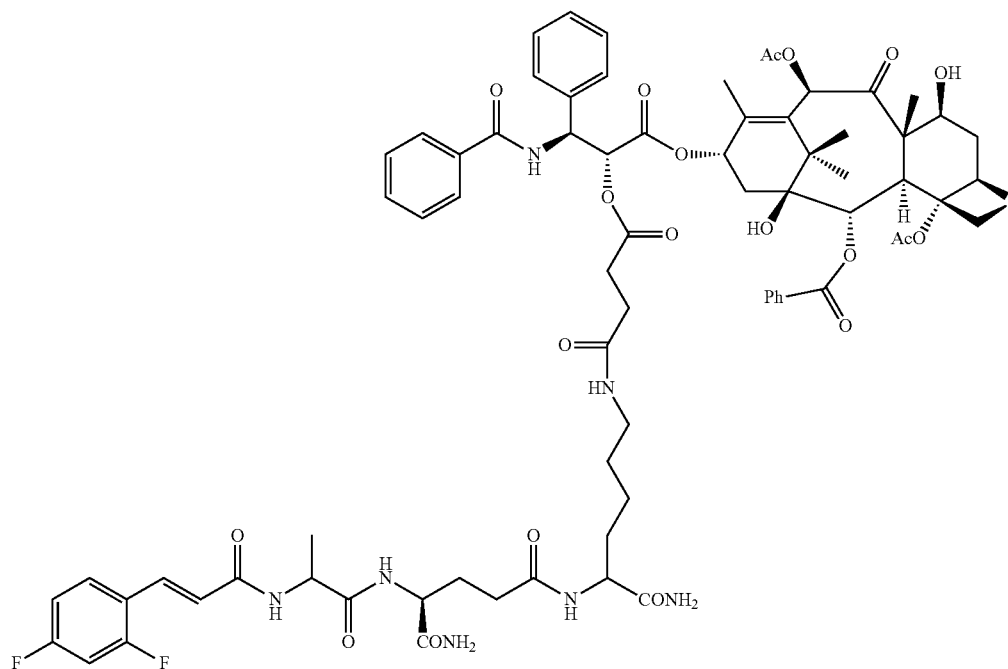
(1H, d, J=8.0 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.34 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.49 (2H, m, ph-o-H), 7.48 (2H, m, ph-m-H), 7.55 (1H, d, J=7.5 Hz, ph-p-H), 7.85 (2H, m, NBz-o-H), 7.46 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.97 (2H, d, J=8.0 Hz, OBz-o-H), 7.65 (2H, d, J=7.5 Hz, OBz-m-H), 7.72 (1H, d, J=7.0 Hz, OBz-p-H), 2.60 (2H, m, 22-H), 2.36 (2H, m, 23-H), 7.84 (1H, m,

56

ESI-MS: 1424.33 [M+H]⁺, 1446.55 [M+Na]⁺.
HR-MS(TOF): 1424.6184 [M+H]⁺, 1446.5996 [M+Na]⁺,
C₇₅H₈₉N₇O₂₁.

Example 26

Liquid-Phase Synthesis of Conjugate MTC-303



25-H), 2.91 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.44 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H_a), 6.96 (1H, s, 32-H_b), 7.30 (1H, m, 32-H_b), 7.86 (1H, m, 33-H), 2.16 (2H, m, 35-H), 1.75 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.12 (1H, m, 37-H), 7.10 (1H, s, 39-H_a), 7.22 (1H, m, 39-H_b), 8.21 (1H, d, J=8.0 Hz, 40-H), 4.37 (1H, m, 42-H), 1.28 (3H, d, J=7.0 Hz, 43-H), 8.31 (1H, d, J=6.5 Hz, 44-H), 6.68 (1H, d, J=15.5 Hz, 46-H), 7.43 (1H, dc J=16.0 Hz, 47-H), 7.57 (1H, m, 49 and 53-H), 7.49 (1H, m, 50 and 52-H), 2.31 (3H, m, 51-CH₃).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.1 (1'-C), 74.6 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.0 (NBz-m-C), 128.3 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.4 (41-C), 48.8 (42-C), 18.1 (43-C), 165.1 (45-C), 120.8 (46-C), 137.4 (47-C), 132.1 (48-C), 129.6 (49 and 53-C), 128.7 (50 and 52-C), 138.9 (51-C), 20.9 (51-CH₃).

IR: 3324.5 (ν_{OH} and ν_{NH}), 3066.3 (ν_{=CH}), 2938.3 (ν_{-CH}), 1740.3, 1724.1, 1657.2 (ν_{C=O}), 1603.9, 1535.1, 1451.8 (ν_{C=C}), 1242.8 (ν_{C-O-C}), 981.3, 709.7 (δ_{=CH}).

953 mg (1.0 eq) pcitaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 510 mg (1.0 eq) muramyl dipeptide analogue MDA-203 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.29 g solid product was obtained through lyophilization. Yield, 89%, m.p.=178~180° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.62 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.13 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.80 (1H, t, J=7.5 Hz, 13-H), 1.45 (1H, m, 14-H_a), 1.77 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.98 (1H, d, J=8.0 Hz, 20-H_a), 4.00 (1H, d, J=8.0 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.10 (3H, s, 10-OCOCH₃), 5.33 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.46 (2H, m, ph-m-H), 7.55 (1H, t, J=7.5 Hz, ph-p-H), 7.82 (2H, m, NBz-o-H), 7.44 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.97 (2H, d, J=8.0 Hz, OBz-o-H), 7.67 (2H, m, OBz-m-H), 7.72 (1H, d, J=8.0 Hz, OBz-p-H), 2.60 (2H, m, 22-H), 2.36 (2H, m, 23-H), 7.82 (1H, m, 25-H), 2.90 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.62 (1H, m,

57

29-H_b), 4.11 (1H, m, 30-H), 7.06 (1H, s, 32-H_a), 7.29 (1H, m, 32-H_b), 7.87 (1H, m, 33-H), 2.14 (2H, m, 35-H), 1.75 (1H, m, 36-H_a), 2.06 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.29 (1H, m, 39-H_b), 8.23 (1H, d, J=8.5 Hz, 40-H), 4.40 (1H, m, 42-H), 1.27 (3H, m, 43-H), 8.47 (1H, d, J=6.5 Hz, 44-H), 6.89 (1H, d, J=17.0 Hz, 46-H), 7.41 (1H, d, J=16.0 Hz, 47-H), 7.34 (1H, td, J=11.5 and 2.0 Hz, 50-H), 7.17 (1H, m, 52-H), 7.74 (1H, m, 53-H).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.6 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.1 (1'-C), 74.6 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.3 (41-C), 48.9 (42-C), 18.1 (43-C), 164.6 (45-C), 124.4 (s, 46-C), 137.4 (s, 47-C), 118.5 (m, 48-C), 161.7 (m, 49-C), 104.6 (t, J=26.1 Hz, 50-C), 163.7 (m, 51-C), 112.4 (d, J=19.9 Hz, 52-C), 130.5 (m, 53-C).

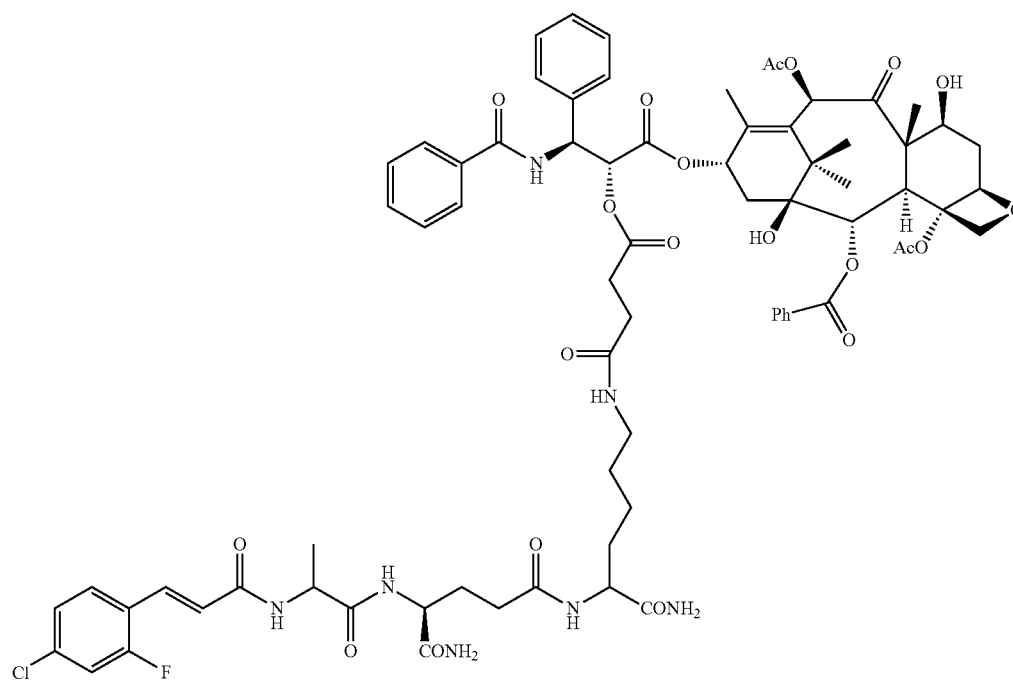
IR: 3309.5 (ν_{OH} and ν_{NH}), 3067.0 (ν_{=CH}), 2945.0 (ν_{-CH}), 1722.0, 1653.8 (ν_{C=O}), 1531.1, 1451.5 (ν_{C=C}), 1239.9 (ν_{C-O-C}), 977.1, 708.3 (δ_{=CH}).

ESI-MS: 1446.03[M+H]⁺, 1468.26 [M+Na]⁺.

HR-MS(TOF): 1446.5877 [M+H]⁺, 1468.5646 [M+Na]⁺, C₇₄H₈₅F₂N₇O₂₁

Example 27

Liquid-Phase Synthesis of Conjugate MTC-304



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were

58

dissolved in DMSO, and stirred at r.t for 4 hours. 526 mg (1.0 eq) muramyl dipeptide analogue MDA-204 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.26 g solid product was obtained through lyophilization. Yield, 86%, m.p.=179~180° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.63 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.5 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.80 (1H, t, J=9.0 Hz, 13-H), 1.45 (1H, m, 14-H_a), 1.78 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.77 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.98 (1H, d, J=8.0 Hz, 20-H), 4.01 (1H, d, J=8.0 Hz, 20-H), 2.23 (3H, s, 4-OCOCH₃), 2.10 (3H, s, 10-OCOCH₃), 5.33 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.45 (2H, m, ph-m-H), 7.55 (1H, m, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.44 (2H, m, NBz-m-H), 7.16 (1H, m, NBz-p-H), 7.97 (2H, d, J=7.0 Hz, OBz-o-H), 7.66 (2H, m, OBz-m-H), 7.74 (1H, d, J=7.5 Hz, OBz-p-H), 2.61 (2H, m, 22-H), 2.35 (2H, m, 23-H), 7.84 (1H, m, 25-H), 2.91 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.21 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, m, 32-H_b), 7.87 (1H, m, 33-H), 2.14 (2H, m, 35-H), 1.75 (1H, m, 36-H_a), 1.98 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.10 (1H, s, 39-H_a), 7.30 (1H, m, 39-H_b), 8.23 (1H, d, J=8.0 Hz, 40-H), 4.40 (1H, m, 42-H), 1.29 (3H, m 43-H), 8.51 (1H, d, J=6.5 Hz, 44-H), 6.85 (1H, d, J=16.0 Hz, 46-H), 7.43 (1H, d, J=16.0 Hz, 47-H), 7.54 (1H, m, 50-H), 7.35 (1H, dd, J=8.5 and 2.0 Hz, 52-H), 7.71 (1H, m, 53-H).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C),

59

57.4 (8-C), 202.3 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.4 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.7 (19-C), 75.2 (20-C), 165.2 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.7, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.7 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.3 (ph-q-C), 127.6 (ph-o-C), 128.3 (ph-m-C), 131.4 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 129.0 (NBz-m-C), 128.1 (NBz-p-C), 134.2 (OBz-q-C), 129.5 (OBz-o-C), 128.6 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 22.9 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.2 (38-C), 172.2 (41-C), 48.9 (42-C), 18.0 (43-C), 164.4 (45-C), 125.3 (m, 46-C), 137.3 (m, 47-C), 122.1 (d, J=11.8 Hz, 48-C), 160.2 (d, J=252.6 Hz, 49-C), 116.7 (d, J=25.5 Hz, 50-C), 134.6 (d, J=10.9 Hz, 51-C), 125.4 (s, 52-C), 130.3 (s, 53-C).

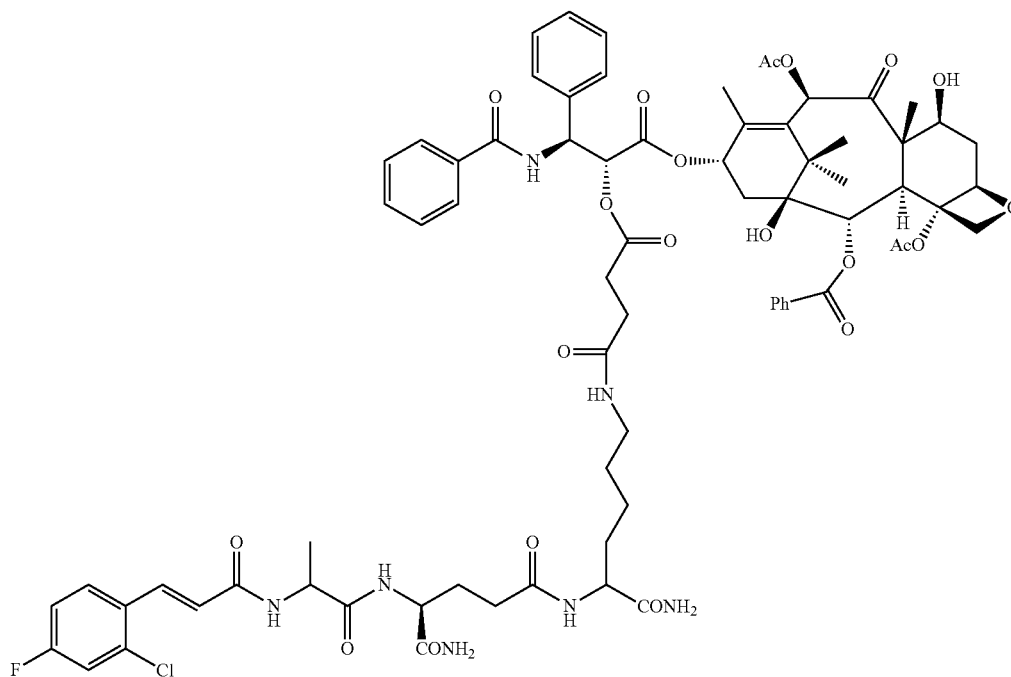
IR: 3324.5 (ν_{OH} and ν_{NH}), 3066.4 (ν_{CH}), 2939.7 (ν_{CH}), 1739.5, 1724.2, 1657.7 (ν_{C=O}), 1604.5, 1534.2, 1451.8 (ν_{C=C}), 1242.6 (ν_{C-O-C}), 981.6, 708.7 (δ_{CH}).

ESI-MS: 1462.59 [M+H]⁺, 1484.93 [M+Na]⁺.

HR-MS(TOF): 1462.5540 [M+H]⁺, 1484.5361 [M+Na]⁺, C₇₄H₈₆ClFN₇O₂₁.

Example 28

Liquid-Phase Synthesis of Conjugate MTC-30



953 mg (1.0 eq) pcitaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 526 mg (1.0 eq) muramyl dipeptide analogue MDA-205 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS col-

60

umn chromatography, 1.18 g solid product was obtained through lypophilization. Yield, 81%, m.p.=171~172° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.63 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.80 (1H, t, J=9.0 Hz, 13-H), 1.46 (1H, m, 14-H_a), 1.77 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.99 (1H, d, J=8.0 Hz, 20-H_a), 4.02 (1H, d, J=8.0 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.10 (3H, s, 10-OCOCH₃), 5.34 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.47 (2H, m, ph-m-H), 7.55 (1H, m, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.44 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.97 (2H, d, J=7.5 Hz, OBz-o-H), 7.66 (2H, m, OBz-m-H), 7.74 (1H, m, OBz-p-H), 2.58 (2H, m, 22-H), 2.33 (2H, t, J=7.0 Hz, 23-H), 7.82 (1H, m, 25-H), 2.91 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.23 (2H, m, 27-H), 1.33 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, m, 32-H_b), 7.86 (1H, m, 33-H), 2.15 (2H, t, J=8.0 Hz, 35-H), 1.71 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.12 (1H, s, 39-H_a), 7.30 (1H, m, 39-H_b), 8.25 (1H, d, J=8.5 Hz, 40-H), 4.41 (1H, m, 42-H), 1.28 (3H, d, J=7.0 Hz, 43-H), 8.45 (1H, d, J=6.5 Hz, 44-H), 6.77 (1H, d, J=16.0 Hz, 46-H), 7.66 (1H, d, J=16.0 Hz, 47-H), 7.54 (1H, m, 50-H), 7.33 (1H, td, J=8.5 and 1.5 Hz, 52-H), 7.76 (1H, m, 53-H).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.1 (1'-C), 74.6 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.1 (NBz-m-C), 128.3 (NBz-p-C), 134.3 (OBz-q-C), 129.6

61

(OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.2 (38-C), 172.2 (41-C), 48.8 (42-C), 18.2 (43-C), 164.2 (45-C), 124.9 (46-C), 137.4 (47-C), 128.8 (48-C), 134.3 (49-C), 115.4 (d, J=21.5 Hz, 50-C), 162.2 (d, J=249.1 Hz, 51-C), 117.2 (d, J=25.1 Hz, 52-C), 129.9 (53-C).

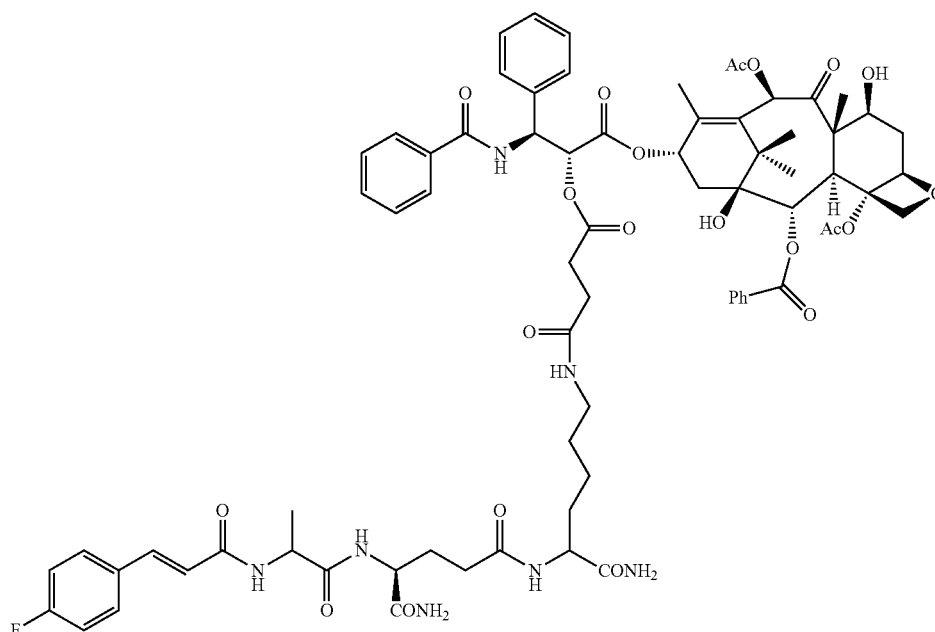
IR: 3315.4 (ν_{OH} and ν_{NH}), 3069.3 ($\nu_{=CH}$), 2935.0 (ν_{-CH}), 1722.8, 1656.5 ($\nu_{C=O}$), 1601.8, 1534.3, 1451.5 ($\nu_{C=C}$), 1239.3 (ν_{C-O-C}), 978.5, 709.7 ($\delta_{=CH}$).

ESI-MS: 1462.89 [M+H]⁺, 1484.21 [M+Na]⁺.

HR-MS(TOF): 1462.5541 [M+H]⁺, 1484.5350 [M+Na]⁺, C₇₄H₈₅ClFN₇O₂₁.

Example 29

Liquid-Phase Synthesis of Conjugate MTC-306



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 492 mg (1.0 eq) muramyl dipeptide analogue MDA-206 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.24 g solid product was obtained through lypophilization. Yield, 87%, m.p.=176~178° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.61 (1H, br.s, 1-OH), 5.41 (1H, d, J=6.0 Hz, 2-H), 3.56 (1H, d, J=5.5 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.30 (1H, m, 6-H_b), 4.11 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, m, 13-H), 1.49 (1H, m, 14-H_a), 1.82 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.76 (3H, s, 18-H), 1.49 (3H, s, 19-H), 3.99 (1H, d, J=5.5 Hz, 20-H_a), 4.00 (1H, d, J=5.5 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.10 (3H, s,

62

10-OCOCH₃), 5.33 (1H, d, J=8.5 Hz, 2'-H), 5.52 (1H, t, J=8.5 Hz, 3'-H), 9.20 (1H, d, J=8.0 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.46 (2H, m, ph-m-H), 7.52 (1H, m, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.19 (1H, m, NBz-p-H), 7.98 (2H, d, J=7.5 Hz, OBz-o-H), 7.67 (2H, m, OBz-m-H), 7.72 (1H, m, OBz-p-H), 2.59 (2H, m, 22-H), 2.35 (2H, m, 23-H), 7.81 (1H, m, 25-H), 2.91 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.94 (1H, s, 32-H_a), 7.28 (1H, m, 32-H_b), 7.85 (1H, m, 33-H), 2.15 (2H, m, 35-H), 1.76 (1H, m, 36-H_a), 1.98 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.09 (1H, s, 39-H_a), 7.28 (1H, m, 39-H_b), 8.20 (1H, d, J=7.5 Hz, 40-H), 4.40 (1H, m, 42-H), 1.26 (3H, m, 43-H), 8.35 (1H, d, J=4.5 Hz, 44-H), 6.79 (1H, d, J=15.5 Hz, 46-H), 7.40 (1H, d, J=15.5 Hz, 47-H), 7.81 (2H, m, 49 and 53-H), 7.39 (2H, m, 50 and 52-H).

¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.3 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4

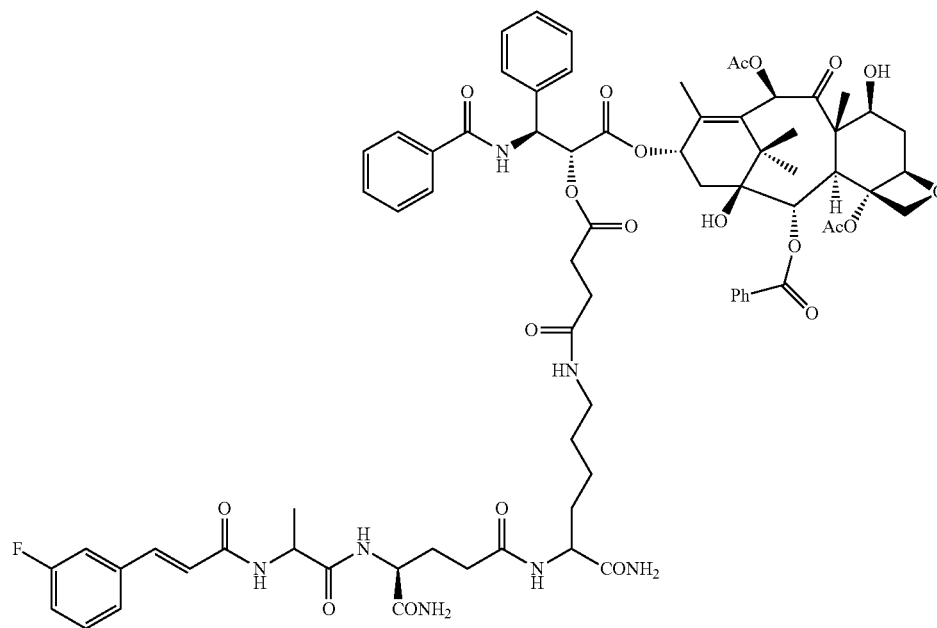
(12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.7 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.8, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.7 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.3 (ph-q-C), 127.6 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 129.0 (NBz-m-C), 128.3 (NBz-p-C), 134.2 (OBz-q-C), 129.5 (OBz-o-C), 128.6 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.2 (38-C), 172.3 (41-C), 48.9 (42-C), 18.1 (43-C), 164.5 (45-C), 123.5 (s, 46-C), 137.4 (s, 47-C), 133.5 (s, 48-C), 130.9 (d, J=8.3 Hz, 49 and 53-C), 116.2 (d, J=21.2 Hz, 50 and 52-C), 162.4 (d, J=242.4 Hz, 51-C).

IR: 3310.1 (ν_{OH} and ν_{NH}), 3063.6 ($\nu_{=CH}$), 2939.5 (ν_{-CH}), 1740.5, 1724.1, 1658.2 ($\nu_{C=O}$), 1582.5, 1536.0, 1450.0 ($\nu_{C=C}$), 1243.5 (ν_{C-O-C}), 978.0, 779.7, 709.5 ($\delta_{=CH}$).

ESI-MS: 1429.41 [M+2H]²⁺, 1451.54 [M+Na+H]²⁺.

HR-MS(TOF): 1428.5950 [M+H]⁺, 1450.5743 [M+Na]⁺, C₇₄H₈₆FN₇O₂₁.

Liquid-Phase Synthesis of Conjugate MTC-307



953 mg (1.0 eq) pectate-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 492 mg (1.0 eq) muramyl dipeptide analogue MDA-207 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.21 g solid product was obtained through lyophilization. Yield, 85%, m.p.=167~168° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.63 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.30 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=7.5 Hz, 13-H), 1.46 (1H, m, 14-H_a), 1.78 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.77 (3H, s, 18-II), 1.48 (3H, s, 19-H), 3.98 (1H, d, J=8.5 Hz, 20-H_a), 4.01 (1H, d, J=8.5 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.32 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.44 (2H, m, ph-m-H), 7.55 (1H, t, J=7.5 Hz, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.19 (1H, m, NBz-p-H), 7.97 (2H, d, J=7.0 Hz, OBz-o-H), 7.65 (2H, t, J=8.0 Hz, OBz-m-H), 7.72 (1H, t, J=7.5 Hz, OBz-p-H), 2.60 (2H, m, 22-H), 2.35 (2H, t, J=7.0 Hz, 23-H), 7.82 (1H, m, 25-H), 2.90 (1H, m, 26-H_a), 3.00 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.33 (2H, m, 28-H), 1.46 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.32 (1H, m, 32-H_b), 7.87 (1H, m, 33-H), 2.15 (2H, t, J=8.0

Hz, 35-H), 1.71 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.30 (1H, m, 39-H_b), 8.22 (1H, d, J=8.0 Hz, 40-H), 4.40 (1H, m, 42-H), 1.26 (3H, d, J=7.0 Hz, 43-H), 8.37 (1H, d, J=6.5 Hz, 44-H), 6.79 (1H, d, J=16.0 Hz, 46-H), 7.49 (1H, d, J=16.0 Hz, 47-H), 7.38 (1H, m, 49-H), 7.22 (1H, m, 51-H), 7.47 (1H, m, 52-H), 7.41 (1H, m, 53-H).

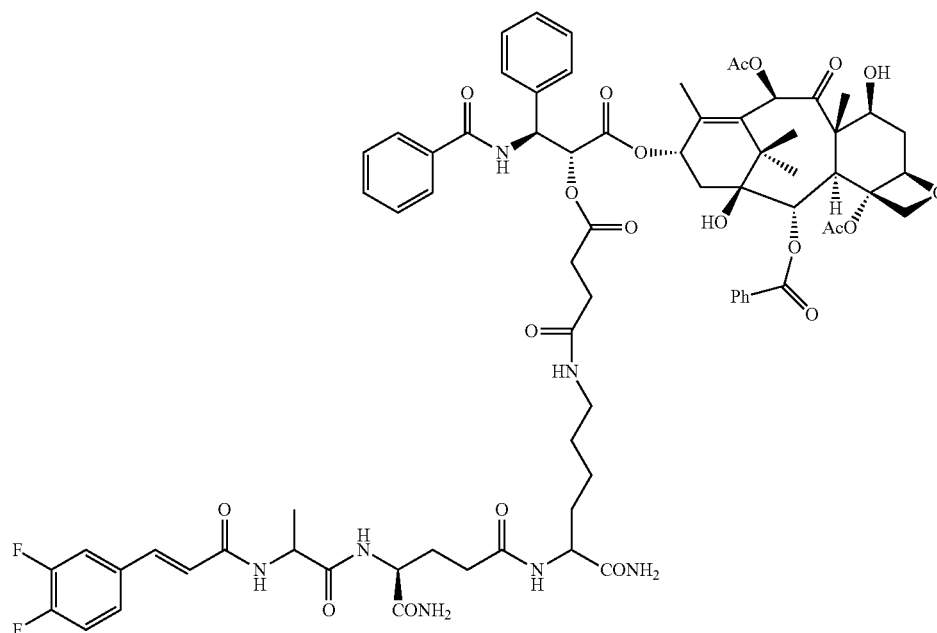
¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.4 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.5 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.5 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.3 (41-C), 48.8 (42-C), 18.1 (43-C), 164.6 (45-C), 123.5 (46-C), 137.5 (47-C), 133.5 (48-C), 113.9 (d, J=21.6 Hz, 49-C), 162.9 (d, J=242.3 Hz, 50-C), 116.7 (d, J=21.0 Hz, 51-C), 130.9 (d, J=8.5 Hz, 52-C), 123.6 (d, J=2.5 Hz, 53-C).

IR: 3320.5 (ν_{OH} and ν_{NH}), 3063.6 (ν_{CH}), 2939.0 (ν_{CH}), 1740.0, 1721.0, 1657.2 (ν_{C=O}), 1582.7, 1536.7, 1450.0 (ν_{C=C}), 1243.6 (ν_{C-O-C}), 979.4, 780.5, 709.5 (δ_{CH}).

ESI-MS: 1429.41 [M+2H]²⁺, 1451.54 [M+Na+H]²⁺.

HR-MS(TOF): 1428.5950 [M+H]⁺, 1450.5736 [M+Na]⁺, C₇₄H₈₆FN₇O₂₁.

Liquid-Phase Synthesis of Conjugate MTC-308



953 mg (1.0 eq) pcitaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC-HC were dissolved in DMSO, and stirred at r.t for 4 hours. 510 mg (1.0 eq) muramyl dipeptide analogue MDA-208 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.14 g solid product was obtained through lypophilization. Yield, 79%, m.p.=167~168° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.62 (1H, br.s, 1-OH), 5.40 (1H, d, J=6.5 Hz, 2-H), 3.56 (1H, d, J=7.0 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.27 (1H, s, 10-H), 5.81 (1H, t, J=8.0 Hz, 13-H), 1.48 (1H, m, 14-H_a), 1.80 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 1.01 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.99 (1H, m, 20-H_a), 4.00 (1H, m, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 2.13 (3H, s, 10-OCOCH₃), 5.32 (1H, d, J=8.5 Hz, 2'-H), 5.51 (1H, t, J=8.5 Hz, 3'-H), 9.21 (1H, d, J=8.5 Hz, 3'-NH), 7.49 (2H, m, ph-o-H), 7.47 (2H, m, ph-m-H), 7.55 (1H, m, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.17 (1H, m, NBz-p-H), 8.06 (2H, d, J=7.0 Hz, OBz-o-H), 7.67 (2H, m, OBz-m-H), 7.72 (1H, d, J=8.0 Hz, OBz-p-H), 2.59 (2H, m, 22-H), 2.35 (2H, m, 23-H), 7.84 (1H, m, 25-H), 2.90 (1H, m, 26-H_a), 3.00 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.31 (2H, m, 28-H), 1.48 (1H, m, 29-H_a), 1.64 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, m, 32-H_b), 7.87 (1H, m, 33-H), 2.14 (2H, m, 35-H), 1.70 (1H, m, 36-H_a), 1.98 (1H, m, 36-H_b), 4.13

(1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.30 (1H, m, 39-H_b), 8.22 (1H, d, J=8.0 Hz, 40-H), 4.40 (1H, m, 42-H), 1.37 (3H, d, J=7.5 Hz, 43-H), 8.34 (1H, d, J=6.5 Hz, 44-H), 6.73 (1H, d, J=15.5 Hz, 46-H), 7.40 (1H, d, J=15.5 Hz, 47-H), 7.67 (1H, m, 50-H), 7.43 (1H, m, 52-H), 7.48 (1H, m, 53-H).

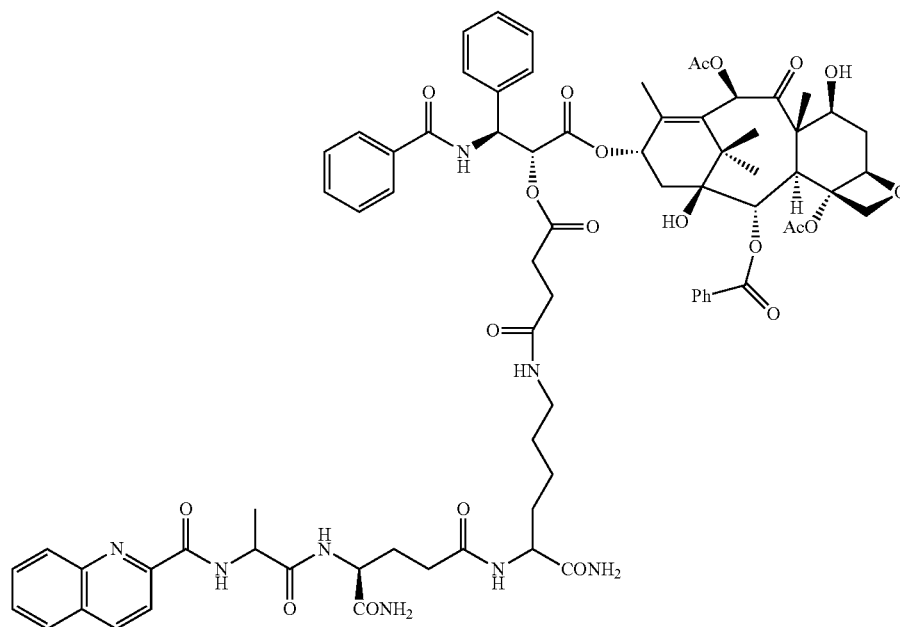
¹³C-NMR (125 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.5 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.2 (1'-C), 74.6 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.5 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.1 (37-C), 173.3 (38-C), 172.3 (41-C), 48.8 (42-C), 18.2 (43-C), 164.7 (45-C), 123.3 (s, 46-C), 137.4 (s, 47-C), 133.3 (m, 48-C), 118.6 (m, 49-C), 151.2 (m, 50-C), 149.3 (m, 51-C), 116.7 (m, 52-C), 125.1 (m, 53-C).

IR: 3306.6 (ν_{OH} and ν_{NH}), 3066.4 (ν_{=CH}), 2932.6 (ν_{-CH}), 1739.8, 1720.2 1658.2 (ν_{C=O}), 1535.1, 1518.5, 1450.2 (ν_{C=C}), 1274.4, 1243.6 (ν_{C-O-C}), 979.7, 775.8, 709.5 (δ_{=CH}).

ESI-MS: 1446.25 [M+H]⁺, 1468.77 [M+Na]⁺.

HR-MS(TOF): 1446.5861 [M+H]⁺, 1468.5651 [M+Na]⁺, C₇₄H₈₅F₂N₇O₂₁.

Liquid-Phase Synthesis of Conjugate MTC-213



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 499 mg (1.0 eq) muramyl dipeptide analogue MDA-113 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.18 g solid product was obtained through lypophilization. Yield, 82%, m.p.=167~168° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.64 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.2 Hz, 2-H), 3.56 (1H, d, J=7.2 Hz, 3-H), 4.91 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.13 (1H, m, 7-H), 4.92 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=9.0 Hz, 13-H), 1.45 (1H, m, 14-H_a), 1.79 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.76 (3H, s, 18-H), 1.51 (3H, s, 19-H), 3.98 (1H, d, J=8.4 Hz, 20-H_a), 4.01 (1H, d, J=8.4 Hz, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.34 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.20 (1H, d, J=9.0 Hz, 3'-NH), 7.48 (2H, d, J=7.8 Hz, ph-o-H), 7.46 (2H, m, ph-m-H), 7.55 (1H, t, J=7.8 Hz, ph-p-H), 7.82 (2H, m, NBz-o-H), 7.43 (2H, m, NBz-m-H), 7.17 (1H, m, NBz-p-H), 7.97 (2H, d, J=7.8 Hz, OBz-o-H), 7.65 (2H, t, J=7.8 Hz, OBz-m-H), 7.72 (1H, t, J=7.8 Hz, OBz-p-H), 2.61 (2H, m, 22-H), 2.35 (2H, t, J=7.2 Hz, 23-H), 7.82 (1H, m, 25-H), 2.90 (1H, m, 26-H_a), 2.98 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.32 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.64 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.29 (1H, s, 32-H_b), 7.87 (1H, m, 33-H), 2.11 (2H, t, J=7.2 Hz, 35-H), 1.71 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b),

4.19 (1H, m, 37-H), 7.09 (1H, s, 39-H_a), 7.29 (1H, s, 39-H_b), 8.16 (1H, d, J=8.4 Hz, 40-H), 4.62 (1H, m, 42-H), 1.27 (3H, d, J=6.6 Hz, 43-H), 8.37 (1H, d, J=7.8 Hz, 44-H), 8.58 (1H, d, J=8.4 Hz, 47-H), 8.92 (1H, d, J=8.4 Hz, 48-H), 7.88 (1H, m, 50-H), 7.49 (1H, m, 51-H), 7.74 (1H, m, 52-H), 8.08 (1H, d, J=8.4 Hz, 53-H).

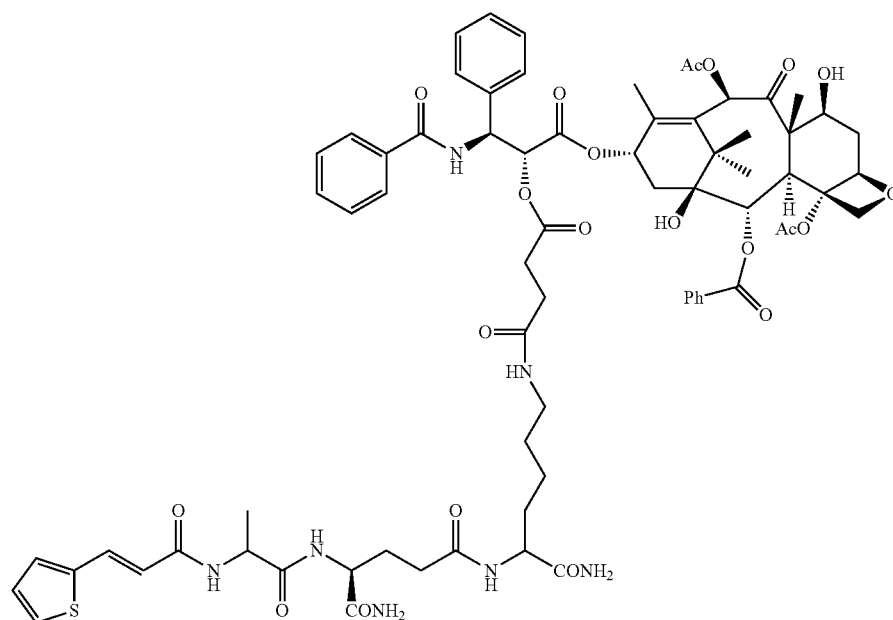
¹³C-NMR (150 MHz, DMSO-d₆): 76.7 (1-C), 74.6 (2-C), 46.1 (3-C), 80.3 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.5 (12-C), 70.7 (13-C), 34.4 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.7, 22.6 (4-OCOCH₃), 168.8, 20.7 (10-OCOCH₃), 169.1 (1'-C), 74.5 (2'-C), 54.0 (3'-C), 166.5 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.5 (NBz-o-C), 128.9 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 171.9 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.8 (27-C), 23.0 (28-C), 31.6 (29-C), 52.3 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.8 (36-C), 52.2 (37-C), 173.2 (38-C), 172.0 (41-C), 48.6 (42-C), 19.0 (43-C), 163.3 (45-C), 149.6 (46-C), 118.5 (47-C), 138.0 (48-C), 128.1 (49-C), 128.6 (50-C), 129.2 (51-C), 130.7 (52-C), 130.3 (53-C), 146.0 (54-C).

IR: 3324.9 (ν_{OH} and ν_{NH}), 2938.5 (ν_{CH}), 1739.6, 1721.9, 1655.0 (ν_{C=O}), 1529.9, 1500.2, 1451.7, 1428.7 (ν_{C=C}), 1371.6, 1242.5, 1177.0, 1070.8 (δ_{CH}), 980.0, 776.8, 708.9 (δ_{CH}).

ESI-MS: 1436.75 [M+2H]²⁺.

HR-MS(TOF): 1435.6001 [M+H]⁺, 1457.5774 [M+Na]⁺, C₇₅H₈₆N₈O₂₁.

Liquid-Phase Synthesis of Conjugate MTC-219



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 480 mg (1.0 eq) muramyl dipeptide analogue MDA-119 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.12 g solid product was obtained through lypophilization. Yield, 79%, m.p.=169~171° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.61 (1H, br.s, 1-OH), 5.41 (1H, d, J=7.0 Hz, 2-H), 3.56 (1H, d, J=8.5 Hz, 3-H), 4.89 (1H, J=10 Hz 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.09 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=9.0 Hz, 13-H), 1.44 (1H, m, 14-H_a), 1.78 (1H, m, 14-H_b), 1.01 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.76 (3H, s, 18-H), 1.49 (3H, s, 19-H), 3.98 (1H, m, 20-H_a), 4.00 (1H, m, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.32 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=8.5 Hz, 3'-H), 9.19 (1H, d, J=8.5 Hz, 3'-NH), 7.48 (2H, m, ph-o-

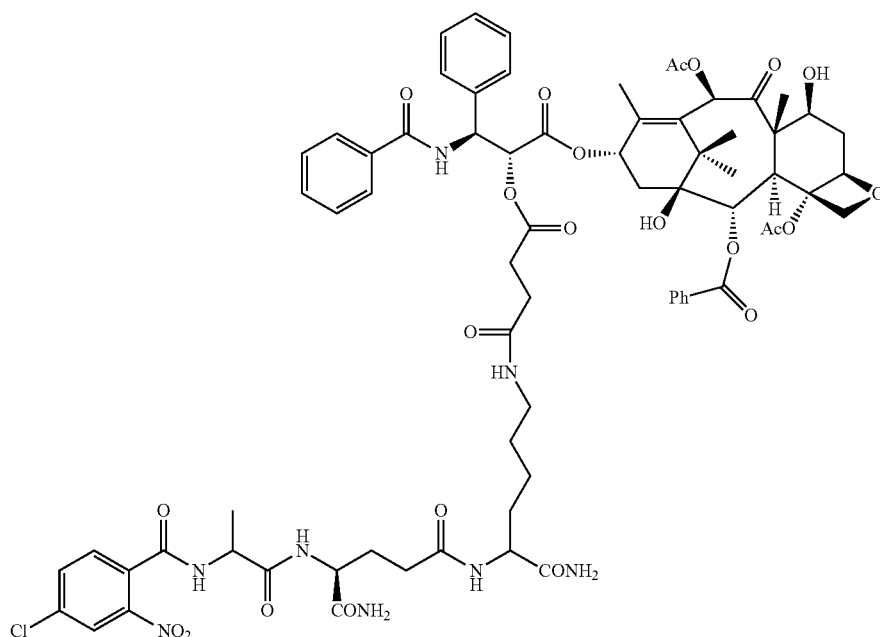
H), 7.43 (2H, m, ph-m-H), 7.55 (1H, m, ph-p-H), 7.84 (2H, m, NBz-o-H), 7.49 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.96 (2H, d, J=8.0 Hz, OBz-o-H), 7.65 (2H, m, OBz-m-H), 7.72 (1H, m, OBz-p-H), 2.63 (2H, m, 22-H), 2.35 (2H, m, 23-H), 7.88 (1H, m, 25-H), 2.93 (1H, m, 26-H_a), 3.21 (1H, m, 26-H_b), 1.23 (2H, m, 27-H), 1.38 (2H, m, 28-H), 1.45 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.10 (1H, m, 30-H), 6.95 (1H, s, 32-H_a), 7.29 (1H, s, 32-H_b), 7.87 (1H, m, 33-H), 2.26 (2H, m, 35-H), 1.76 (1H, m, 36-H_a), 1.95 (1H, m, 36-H_b), 4.12 (1H, m, 37-H), 7.03 (1H, s, 39-H_a), 7.29 (1H, s, 39-H_b), 8.24 (1H, d, J=8.0 Hz, 40-H), 4.37 (1H, m, 42-H), 1.25 (3H, m, 43-H), 8.39 (1H, m, 44-H), 6.97 (1H, d, J=15.0 Hz, 46-H), 7.45 (1H, d, J=15.0 Hz, 47-H), 8.17 (1H, m, 50-H), 7.59 (1H, m, 51-H), 7.72 (1H, m, 52-H).

IR: 3331.9 (ν_{OH} and ν_{NH}), 2963.6, 2936.7 (ν_{CH}), 1739.2, 1712.5, 1649.9 (ν_{C=O}), 1538.4, 1452.3, 1438.2 (ν_{C-C}), 1370.7, 1243.8, 1172.5, 1144.1 (δ_{CH}), 980.0, 833.2, 706.6 (δ_{=CH}).

ESI-MS: 1417.21 [M+2H]²⁺.

HR-MS(TOF): 1416.5542 [M+H]⁺, 1438.5365 [M+Na]⁺, C₇₂H₈₅N₇O₂₁S.

Liquid-Phase Synthesis of Conjugate MTC-230



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t for 4 hours. 553 mg (1.0 eq) muramyl dipeptide analogue MDA-130 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.28 g solid product was obtained through lypophilization. Yield, 86%, m.p.=172~173° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.62 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.2 Hz, 2-H), 3.56 (1H, d, J=7.2 Hz, 3-H), 4.90 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=9.0 Hz, 13-H), 1.51 (1H, m, 14-H_a), 1.79 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.48 (3H, s, 19-H), 3.98 (1H, d, J=7.8 Hz, 20-H_a), 4.00 (1H, d, J=7.8 Hz, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.33 (1H, d, J=7.8 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.20 (1H, d, J=9.0 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.43 (2H, m, ph-m-H), 7.55 (1H, t, J=7.8 Hz, ph-p-H), 7.83 (2H, m, NBz-o-H), 7.42 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.98 (2H, d, J=7.2 Hz, OBz-o-H), 7.66 (2H, t, J=7.2 Hz, OBz-m-H), 7.72 (1H, t, J=7.2 Hz, OBz-p-H), 2.60 (2H, m, 22-H), 2.35 (2H, m, 23-H), 7.82 (1H, m, 25-H), 2.91 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.30 (2H, m, 28-H), 1.44 (1H, m, 29-H_a), 1.62 (1H,

m, 29-H_b), 4.11 (1H, m, 30-H), 6.95 (1H, s, 32-H_a), 7.29 (1H, s, 32-H_b), 7.87 (1H, m, 33-H), 2.17 (2H, t, J=7.8 Hz, 35-H), 1.72 (1H, m, 36-H_a), 1.97 (1H, m, 36-H_b), 4.12 (1H, m, 37-H), 7.09 (1H, s, 39-H_a), 7.29 (1H, s, 39-H_b), 8.16 (1H, d, J=7.8 Hz, 40-H), 4.46 (1H, m, 42-H), 1.30 (3H, d, J=6.6 Hz, 43-H), 8.52 (1H, d, J=6.6 Hz, 44-H), 7.70 (1H, m, 47-H), 7.84 (1H, m, 48-H), 8.97 (1H, m, 50-H).

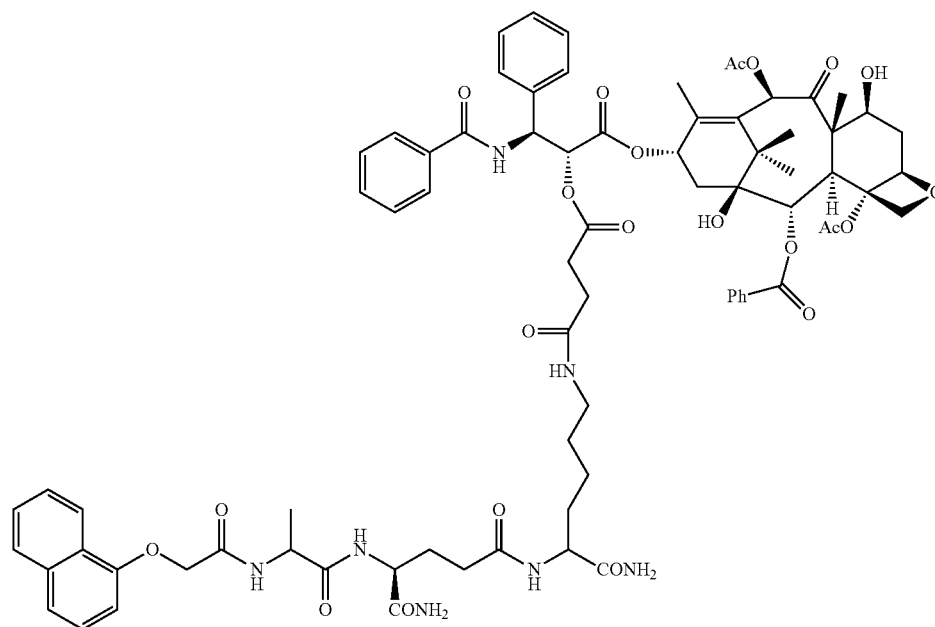
¹³C-NMR (150 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.4 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.3 (16-C), 21.5 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 169.6, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.4 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.7 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 129.0 (NBz-m-C), 128.3 (NBz-p-C), 134.3 (OBz-q-C), 129.5 (OBz-o-C), 128.6 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 22.9 (28-C), 31.5 (29-C), 52.4 (30-C), 173.9 (31-C), 171.6 (34-C), 31.7 (35-C), 27.8 (36-C), 52.1 (37-C), 173.1 (38-C), 173.2 (41-C), 48.8 (42-C), 19.7 (43-C), 164.4 (45-C), 131.5 (46-C), 130.6 (47-C), 134.5 (48-C), 147.7 (49-C), 124.0 (50-C), 149.7 (51-C).

IR: 3277.6 (ν_{OH} and ν_{NH}), 3065.0 (ν_{CH}), 2973.2, 2936.4 (ν_{CH}), 1719.3, 1646.9, 1629.8 (ν_{C=O}), 1537.1, 1452.0 (ν_{C=C}), 1350.0, 1240.9, 1151.2 (δ_{CH}), 978.4, 895.0, 706.3 (δ_{CH}).

ESI-MS: 1463.70 [M+H]⁺.

HR-MS(TOF): 1463.5293 [M+H]⁺, 1485.5120 [M+Na]⁺, C₇₂H₈₃ClN₈O₂₃.

Liquid-Phase Synthesis of Conjugate MTC-233



953 mg (1.0 eq) pcatiaxel-2'-O-succinic acid monoester, 115 mg (1.0 eq) HOSu and 192 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and stirred at r.t. for 4 hours. 528 mg (1.0 eq) muramyl dipeptide analogue MDA-133 was sparingly added to the mixture in a few portions. The pH of the mixture was adjusted to 7~8 with N-methyl morpholine, and continued to stir for 4 hours. After the completion of the reaction, plenty of water was added to the mixture, and white solid precipitated. The mixture was filtered and the crude product was obtained. The crude product was purified by ODS column chromatography, 1.17 g solid product was obtained through lypophilization. Yield, 80%, m.p.=155~156° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.61 (1H, br.s, 1-OH), 5.40 (1H, d, J=7.2 Hz, 2-H), 3.56 (1H, d, J=7.2 Hz, 3-H), 4.90 (1H, m, 5-H), 1.62 (1H, m, 6-H_a), 2.31 (1H, m, 6-H_b), 4.12 (1H, m, 7-H), 4.91 (1H, m, 7-OH), 6.28 (1H, s, 10-H), 5.81 (1H, t, J=9.0 Hz, 13-H), 1.48 (1H, m, 14-H_a), 1.79 (1H, m, 14-H_b), 0.98 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.75 (3H, s, 18-H), 1.49 (3H, s, 19-H), 3.98 (1H, d, J=8.4 Hz, 20-H_a), 4.00 (1H, d, J=8.4 Hz, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 2.09 (3H, s, 10-OCOCH₃), 5.33 (1H, d, J=9.0 Hz, 2'-H), 5.52 (1H, t, J=9.0 Hz, 3'-H), 9.19 (1H, d, J=9.0 Hz, 3'-NH), 7.48 (2H, m, ph-o-H), 7.43 (2H, m, ph-m-H), 7.56 (1H, m, ph-p-H), 7.83 (2H, m, NBz-o-H), 7.42 (2H, m, NBz-m-H), 7.18 (1H, m, NBz-p-H), 7.97 (2H, d, J=7.2 Hz, OBz-o-H), 7.66 (2H, m, OBz-m-H), 7.72 (1H, m, OBz-p-H), 2.60 (2H, m, 22-H), 2.35 (2H, t, J=7.2 Hz, 23-H), 7.82 (1H, m, 25-H), 2.90 (1H, m, 26-H_a), 2.96 (1H, m, 26-H_b), 1.22 (2H, m, 27-H), 1.33 (2H, m, 28-H), 1.44 (1H, m, 29-H_a), 1.62 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.94 (1H, s, 32-H_a), 7.37 (1H, s, 32-H_b), 7.87 (1H, m, 33-H), 2.15 (2H, t, J=7.8 Hz, 35-H), 1.70 (1H, m,

36-H_a), 1.97 (1H, m, 36-H_b), 4.12 (1H, m, 37-H), 7.09 (1H, s, 39-H_a), 7.32 (1H, s, 39-H_b), 8.21 (1H, d, J=8.4 Hz, 40-H), 4.43 (1H, m, 42-H), 1.29 (3H, d, J=6.6 Hz, 43-H), 8.28 (1H, d, J=7.8 Hz, 44-H), 4.73 (1H, s, 46-H), 6.20 (1H, d, J=7.8 Hz, 48-H), 7.32 (1H, m, 49-H), 7.38 (1H, m, 50-H), 7.97 (1H, m, 52-H), 7.49 (1H, m, 53-H), 7.54 (1H, m, 54-H), 8.30 (1H, m, 55-H).

¹³C-NMR (150 MHz, DMSO-d₆): 76.7 (1-C), 74.5 (2-C), 46.1 (3-C), 80.2 (4-C), 83.6 (5-C), 36.5 (6-C), 70.4 (7-C), 57.4 (8-C), 202.3 (9-C), 74.7 (10-C), 133.3 (11-C), 139.4 (12-C), 70.7 (13-C), 34.4 (14-C), 42.9 (15-C), 26.3 (16-C), 21.4 (17-C), 13.9 (18-C), 9.8 (19-C), 75.3 (20-C), 165.2 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.8, 20.6 (10-OCOCH₃), 169.1 (1'-C), 74.4 (2'-C), 54.0 (3'-C), 166.4 (3'-NHCO), 137.4 (ph-q-C), 127.7 (ph-o-C), 128.3 (ph-m-C), 131.5 (ph-p-C), 129.9 (NBz-q-C), 127.4 (NBz-o-C), 129.0 (NBz-m-C), 128.2 (NBz-p-C), 134.3 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.5 (OBz-p-C), 172.0 (21-C), 28.8 (22-C), 29.5 (23-C), 170.0 (24-C), 38.5 (26-C), 28.7 (27-C), 22.9 (28-C), 31.6 (29-C), 52.4 (30-C), 173.9 (31-C), 171.5 (34-C), 31.7 (35-C), 27.7 (36-C), 52.2 (37-C), 173.2 (38-C), 173.3 (41-C), 48.2 (42-C), 18.4 (43-C), 167.2 (45-C), 67.2 (46-C), 153.1 (47-C), 105.7 (48-C), 126.1 (49-C), 120.7 (50-C), 134.0 (51-C), 127.6 (52-C), 126.1 (53-C), 125.4 (54-C), 121.7 (55-C), 127.4 (56-C).

IR: 3289.3 (ν_{OH} and ν_{NH}), 3065.7 (ν_{CH}), 2937.8 (ν_{CH}), 1739.5, 1720.9, 1647.6 (ν_{C=O}), 1577.5, 1537.2, 1450.4 (ν_{C=C}), 1265.1, 1239.5, 1154.1 (δ_{CH}), 905.9, 853.3, 792.9, 771.3, 707.4 (δ_{CH}).

ESI-MS: 1465.32 [M+2H]²⁺.

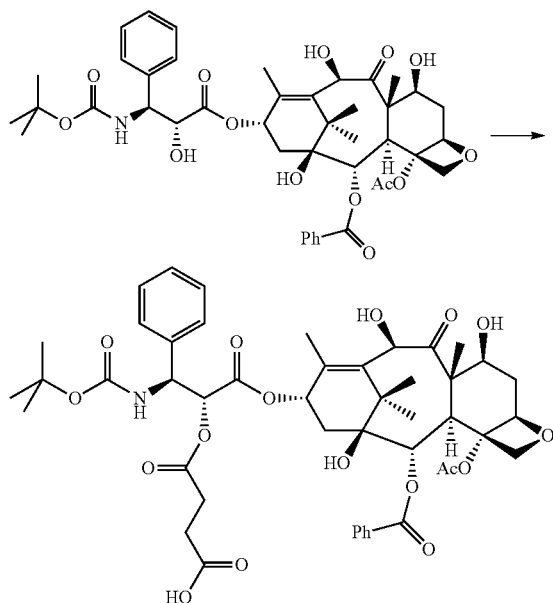
HR-MS(TOF): 1464.6128 [M+H]⁺, 1486.5942 [M+Na]⁺, C₇₇H₈₉N₇O₂₂.

75

Example 36

Liquid-phase synthesis of docetaxel-2'-O-succinic acid monoester

The synthetic route was shown as below:



Reagents and conditions: succinic anhydride, DMAP, r.t, 2 h.

8.07 g (1.0 eq) docetaxel, 1.2 g (1.2 eq) succinic anhydride and 0.61 g (0.5 eq) DMAP were dissolved in DMF, and the mixture was stirred at r.t for 2 hours. After the completion of the reaction, the mixture was diluted with DCM, and the DCM layer was washed with 2 N HCl aqueous solution 3 times, and water for 1 time in sequence. The DCM layer was separated, and evaporated under vacuum. Large amount of

76

water was added to the residue, and white solids precipitated. The mixture was filtered, and 7.9 g target compound was obtained through lyophilization. Yield 87%, m.p.=181~182° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.43 (1H, br.s, 1-OH), 5.39 (1H, d, J=7.2 Hz, 2-H), 3.62 (1H, d, J=7.2 Hz, 3-H), 4.89 (1H, d, J=9.6 Hz, 5-H), 1.62 (1H, m, 6-H_a), 2.22 (1H, d, J=9.6 Hz, 6-H_b), 4.04 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.77 (1H, t, J=9.0 Hz, 13-H), 1.62 (1H, m, 14-H_a), 1.85 (1H, dd, J=15.0 and 9.0 Hz, 14-H_b), 0.97 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.73 (3H, s, 18-H), 1.51 (3H, s, 19-H), 3.98 (1H, d, J=9.0 Hz, 20-H_a), 4.02 (1H, d, J=9.0 Hz, 20-H_b), 2.26 (3H, s, 4-OCOCH₃), 5.06 (1H, m, 2'-H), 5.07 (1H, m, 3'-H), 7.86 (1H, d, J=8.4 Hz, 3'-NH), 7.35 (2H, d, J=7.8 Hz, ph-o-H), 7.40 (2H, t, J=7.8 Hz, ph-m-H), 7.17 (1H, t, J=7.8 Hz, ph-p-H), 7.97 (2H, d, J=7.8 Hz, OBz-o-H), 7.63 (2H, d, J=7.8 Hz, OBz-m-H), 7.71 (1H, d, J=7.8 Hz, OBz-p-H), 1.37 (9H, s, —C(CH₃)₃), 2.50 (2H, m, —CH₂—CH₂—COOH), 2.60 (2H, m, —CH₂—CH₂—COOH), 12.23 (1H, br.s, —CH₂—COOH).

¹³C-NMR (150 MHz, DMSO-d₆): 76.8 (1-C), 74.8 (2-C), 46.0 (3-C), 80.3 (4-C), 83.7 (5-C), 36.5 (6-C), 70.8 (7-C), 56.9 (8-C), 209.3 (9-C), 73.7 (10-C), 135.9 (11-C), 136.8 (12-C), 71.7 (13-C), 34.7 (14-C), 42.9 (15-C), 26.4 (16-C), 20.8 (17-C), 13.7 (18-C), 9.8 (19-C), 75.4 (20-C), 165.3 (2-OCO), 169.5, 22.5 (4-OCOCH₃), 168.3 (1'-C), 75.1 (2'-C), 57.4 (3'-C), 155.2 (3'-NHCO), 78.5, 28.1 (—C(CH₃)₃), 137.4 (ph-q-C), 127.4 (ph-o-C), 128.5 (ph-m-C), 128.0 (ph-p-C), 130.0 (OBz-q-C), 129.5 (OBz-o-C), 128.7 (OBz-m-C), 133.4 (OBz-p-C), 171.5, 28.4, 28.5, 172.9 (—CO—CH₂—CH₂—COOH).

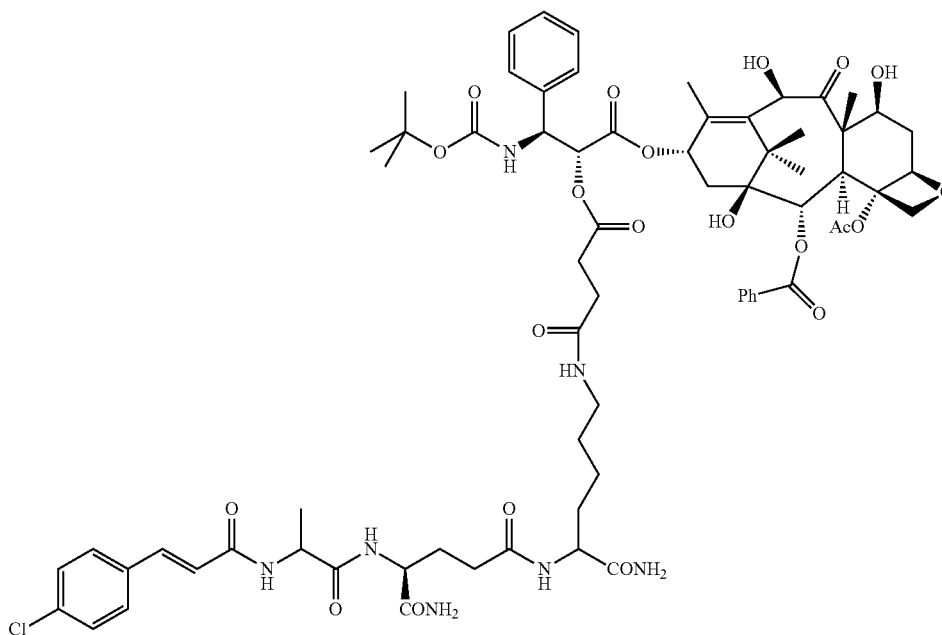
ESI-MS: 930.31 [M+Na]⁺.HR-MS(TOF): 930.3507 [M+Na]⁺, C₄₇H₅₇NO₁₇.

Examples 37-43

Liquid-Phase Synthesis of Conjugate MDC

Example 37

Liquid-Phase Synthesis of Conjugate MDC 400



77

90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 50.8 mg (1.0 eq) of muramyl dipeptide analogue MDA

was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7-8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 124 mg solid product was obtained through lypophilization. Yield 89%, m.p.=180~181° C.

¹H-NMR (600 MHz, DMSO-d₆): 4.41 (1H, br.s, 1-OH), 5.39 (1H, d, J=6.6 Hz, 2-H), 3.62 (1H, d, J=6.6 Hz, 3-H), 4.89 (1H, d, J=10.2 Hz, 5-H), 1.66 (1H, m, 6-H_a), 2.26 (1H, m, 6-H_b), 4.04 (1H, m, 7-H), 5.07 (1H, s, 10-H), 5.77 (1H, t, J=9.0 Hz, 13-H), 1.64 (1H, m, 14-H_a), 1.82 (1H, dd, J=15.6 and 9.0 Hz, 14-H_b), 0.96 (3H, s, 16-H), 0.97 (3H, s, 17-H), 1.68 (3H, s, 18-H), 1.50 (3H, s, 19-H), 3.99 (1H, m, 20-H_a), 4.01 (1H, d, J=9.0 Hz, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 5.04 (1H, m, 2'-H), 5.06 (1H, m, 3'-H), 7.86 (1H, m, 3'-NH), 7.30 (2H, m, ph-o-H), 7.35 (2H, d, J=7.8 Hz, ph-m-H), 7.16 (1H, t, J=7.2 Hz, ph-p-H), 7.97 (2H, d, J=7.8 Hz, OBz-o-H), 7.64

78

(12-C), 71.1 (13-C), 34.7 (14-C), 42.9 (15-C), 26.5 (16-C), 20.8 (17-C), 13.6 (18-C), 9.8 (19-C), 75.3 (20-C), 165.3 (2-OCO), 169.6, 22.5 (4-OCOCH₃), 168.9 (1'-C), 75.0 (2'-C), 55.1 (3'-C), 155.2 (3'-NHCO), 78.5, 28.1 (—C(CH₃)₃), 137.5 (ph-q-C), 127.4 (ph-o-C), 128.5 (ph-m-C), 128.0 (ph-p-C), 130.0 (OBz-q-C), 129.6 (OBz-o-C), 128.7 (OBz-m-C), 133.4 (OBz-p-C), 171.9 (21-C), 28.9 (22-C), 29.6 (23-C), 170.0 (24-C), 38.5 (26-C), 28.9 (27-C), 23.0 (28-C), 31.4 (29-C), 52.1 (30-C), 174.1 (31-C), 171.6 (34-C), 31.7 (35-C), 27.7 (36-C), 52.4 (37-C), 173.4 (38-C), 172.3 (41-C), 48.8 (42-C), 18.1 (43-C), 164.7 (45-C), 122.7 (46-C), 137.6 (47-C), 133.8 (48-C), 129.0 (49 and 53-C), 129.2 (50 and 52-C), 134.0 (51-C).

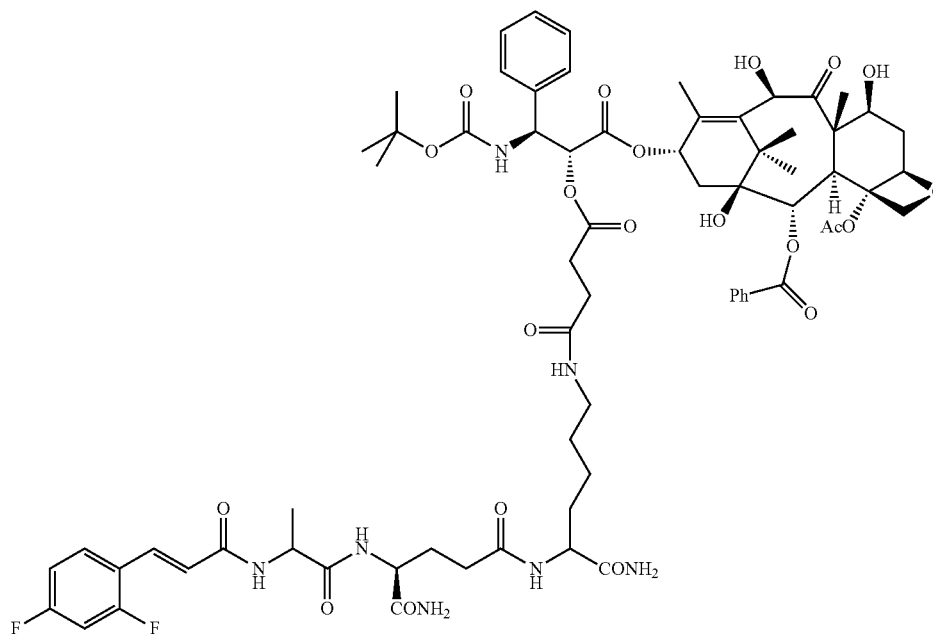
IR: 3320.6 (ν_{OH} and ν_{NH}), 2976.8, 2933.5 (ν_{CH}), 1739.7, 1658.6 (ν_{C=O}), 1531.5, 1496.5, 1452.4 (ν_{C=C}), 1246.2 (ν_{C-O-C}), 983.5, 707.9 (δ_{CH}).

ESI-MS: 1398.14 [M+H]⁺, 1420.32 [2M+Na]⁺.

HR-MS(TOF): 1398.5791 [M+H]⁺, 1420.5609 [M+Na]⁺, C₇₀H₈₈ClN₇O₂₁.

Example 38

Liquid-Phase Synthesis of Conjugate MDC 403



(2H, t, J=7.8 Hz, OBz-m-H), 7.71 (1H, t, J=7.2 Hz, OBz-p-H), 1.36 (9H, s, —C(CH₃)₃), 2.59 (2H, m, 22-H), 2.36 (2H, m, 23-H), 7.83 (1H, m, 25-H), 2.92 (1H, m, 26-H_a), 3.00 (1H, m, 26-H_b), 1.21 (2H, m, 27-H), 1.27 (2H, m, 28-H), 1.52 (1H, m, 29-H_a), 1.63 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, s, 32-H_b), 7.90 (1H, m, 33-H), 2.15 (2H, m, 35-H), 1.72 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.02 (1H, s, 39-H_a), 7.30 (1H, s, 39-H_b), 8.29 (1H, m, 40-H), 4.38 (1H, m, 42-H), 1.26 (3H, d, J=6.6 Hz, 43-H), 8.38 (1H, d, J=6.6 Hz, 44-H), 6.75 (1H, d, J=16.2 Hz, 46-H), 7.37 (1H, d, J=16.3 Hz, 47-H), 7.57 (2H, d, J=8.4 Hz, 49 and 53-H), 7.46 (2H, d, J=8.4 Hz, 50 and 52-H).

¹³C-NMR (150 MHz, DMSO-d₆): 76.8 (1-C), 74.8 (2-C), 46.1 (3-C), 80.3 (4-C), 83.7 (5-C), 36.5 (6-C), 70.7 (7-C), 57.0 (8-C), 209.3 (9-C), 73.7 (10-C), 136.0 (11-C), 136.8

90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 51 mg (1.0 eq) of muramyl dipeptide analogue MDA-203 was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7-8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 114 mg solid product was obtained through lypophilization. Yield 80%, m.p.=165~166° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.45 (1H, br.s, 1-OH), 5.44 (1H, d, J=6.0 Hz, 2-H), 3.64 (1H, d, J=6.0 Hz, 3-H), 4.89

79

(1H, m, 5-H), 1.66 (1H, m, 6-H_a), 2.25 (1H, m, 6-H_b), 4.03 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.80 (1H, m, 13-H), 1.64 (1H, m, 14-H_a), 1.82 (1H, m, 14-H_b), 0.96 (3H, s, 16-H), 0.96 (3H, s, 17-H), 1.68 (3H, s, 18-H), 1.52 (3H, s, 19-H), 3.99 (1H, m, 20-H_a), 4.01 (1H, m, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 5.04 (1H, m, 2'-H), 5.06 (1H, m, 3'-H), 7.86 (1H, m, 3'-NH), 7.31 (2H, m, ph-o-H), 7.38 (2H, min. ph-m-H), 7.19 (1H, m, ph-p-H), 7.99 (2H, d, J=6.5 Hz, OBz-o-H), 7.66 (2H, m, OBz-m-H), 7.72 (1H, m, OBz-p-H), 1.39 (9H, s,

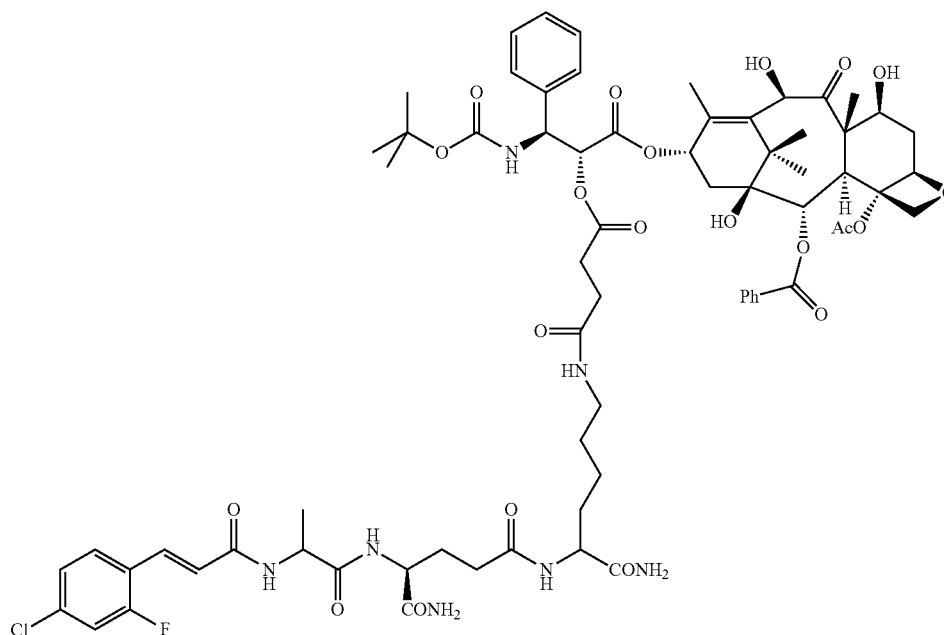
80

ESI-MS: 1400.98 [M+H]⁺, 1422.43 [M+Na]⁺.

HR-MS(TOF): 1400.6008 [M+H]⁺, 1422.5824 [M+Na]⁺, C₇₀H₈₇F₂N₇O₂₁.

Example 39

Liquid-Phase Synthesis of Conjugate MDC 404



—C(CH₃)₃), 2.62 (2H, m, 22-H), 2.39 (2H, m, 23-H), 7.83 (1H, m, 25-H), 3.01 (2H, br.s, 26-H), 1.21 (2H, m, 27-H), 1.29 (2H, m, 28-H), 1.52 (1H, br.s, 29-H_a), 1.63 (1H, br.s, 29-H_b), 4.14 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.31 (1H, s, 32-H_b), 7.90 (1H, m, 33-H), 2.17 (2H, m, 35-H), 1.70 (1H, m, 36-H_a), 1.99 (1H, m, 36-H_b), 4.13 (1H, m, 37-H), 7.02 (1H, s, 39-H_a), 7.30 (1H, s, 39-H_b), 8.22 (1H, m, 40-H), 4.38 (1H, m, 42-H), 1.26 (3H, m, 43-H), 8.47 (1H, d, J=6.0 Hz, 44-H), 6.82 (1H, d, J=16.0 Hz, 46-H), 7.37 (1H, m, 47-H), 7.18 (1H, m, 51-H), 7.70 (1H, m, 53-H).

¹³C-NMR (125 MHz, DMSO-d₆): 77.2 (1-C), 75.2 (2-C), 46.4 (3-C), 80.8 (4-C), 84.2 (5-C), 36.9 (6-C), 71.2 (7-C), 57.4 (8-C), 209.3 (9-C), 74.2 (10-C), 136.0 (11-C), 136.8 (12-C), 71.2 (13-C), 35.2 (14-C), 43.3 (15-C), 26.9 (16-C), 21.2 (17-C), 14.1 (18-C), 10.3 (19-C), 75.3 (20-C), 165.1 (2-OCO), 170.5, 22.9 (4-OCOCH₃), 168.9 (1'-C), 75.0 (2'-C), 55.6 (3'-C), 155.2 (3'-NHCO), 79.0, 28.1 (—C(CH₃)₃), 137.5 (ph-q-C), 127.9 (ph-o-C), 128.5 (ph-m-C), 128.0 (ph-p-C), 130.0 (OBz-q-C), 129.2 (OBz-o-C), 128.7 (OBz-m-C), 133.4 (OBz-p-C), 172.0 (21-C), 28.6 (22-C), 29.3 (23-C), 170.0 (24-C), 39.0 (26-C), 28.6 (27-C), 23.4 (28-C), 31.4 (29-C), 52.1 (30-C), 174.1 (31-C), 171.6 (34-C), 31.7 (35-C), 27.7 (36-C), 52.6 (37-C), 173.7 (38-C), 172.3 (41-C), 49.4 (42-C), 18.5 (43-C), 164.7 (45-C), 122.7 (46-C), 137.6 (47-C), 118.5 (m, 48-C), 161.7 (m, 49-C), 104.6 (m, 50-C), 163.7 (m, 51-C), 112.4 (m, 52-C), 130.5 (m, 53-C).

IR: 3323.9 (ν_{OH} and ν_{NH}), 2977.6, 2937.6 (ν_{CH}), 1739.5, 1659.3 (ν_{C=O}), 1532.5, 1504.2, 1452.5 (ν_{C=C}), 1368.2, 1272.7, 1246.8, 1161.2, 1069.2 (δ_{CH}), 983.0, 852.5, 708.8 (δ_{=CH}).

90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 52.6 mg (1.0 eq) of muramyl dipeptide analogue MDA-204 was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7~8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 116 mg solid product was obtained through lyophilization. Yield, 82%, m.p.=175~176° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.42 (1H, br.s, 1-OH), 5.41 (1H, d, J=7.0 Hz, 2-H), 3.65 (1H, d, J=7.0 Hz, 3-H), 4.90 (1H, m, 5-H), 1.63 (1H, m, 6-H_a), 2.28 (1H, m, 6-H_b), 4.05 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.78 (1H, t, J=8.5 Hz, 13-H), 1.63 (1H, m, 14-H_a), 1.83 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 1.02 (3H, s, 17-H), 1.68 (3H, s, 18-H), 1.51 (3H, s, 19-H), 4.00 (1H, m, 20-H_a), 4.02 (1H, m, 20-H_b), 2.23 (3H, s, 4-OCOCH₃), 5.02 (1H, m, 2'-H), 5.09 (1H, m, 3'-H), 7.86 (1H, m, 3'-NH), 7.30 (2H, m, ph-o-H), 7.37 (2H, m, ph-m-H), 7.18 (1H, m, ph-p-H), 7.99 (2H, d, J=7.5 Hz, OBz-o-H), 7.65 (2H, m, OBz-m-H), 7.71 (1H, m, OBz-p-H), 1.36 (9H, s, —C(CH₃)₃), 2.61 (2H, m, 22-H), 2.37 (2H, m, 23-H), 7.83 (1H, m, 25-H), 3.00 (1H, m, 26-H_a), 3.01 (1H, m, 26-H_b), 1.20 (2H, m, 27-H), 1.29 (2H, m, 28-H), 1.52 (1H, m, 29-H_a), 1.63 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.96 (1H, s, 32-H_a), 7.30 (1H, s, 32-H_b), 7.88 (1H, m, 33-H), 2.16 (2H, m, 35-H), 1.74 (1H, m, 36-H_a), 2.00 (1H, m, 36-H_b), 4.13 (1H, m,

(12-C), 71.6 (13-C), 35.2 (14-C), 43.3 (15-C), 26.9 (16-C), 21.2 (17-C), 14.1 (18-C), 10.3 (19-C), 75.9 (20-C), 165.7 (2-OCO), 170.0, 22.9 (4-OCOCH₃), 169.4 (1'-C), 75.5 (2'-C), 55.5 (3'-C), 155.7 (3'-NHCO), 78.9, 28.2 (—C(CH₃)₃), 137.3 (ph-q-C), 127.9 (ph-o-C), 129.0 (ph-m-C), 129.1 (ph-p-C), 130.5 (OBz-q-C), 130.0 (OBz-o-C), 129.1 (OBz-m-C), 133.6

83

(OBz-p-C), 172.0 (21-C), 29.3 (22-C), 30.1 (23-C), 170.4 (24-C), 38.5 (26-C), 28.6 (27-C), 23.4 (28-C), 32.1 (29-C), 52.6 (30-C), 174.4 (31-C), 172.3 (34-C), 32.2 (35-C), 26.9 (36-C), 52.8 (37-C), 173.7 (38-C), 172.7 (41-C), 49.3 (42-C), 18.7 (43-C), 164.7 (45-C), 125.4 (46-C), 133.9 (47-C), 129.2 (48-C), 134.6 (49-C), 115.8 (d, J=21.6 Hz, 50-C), 162.7 (d, J=249.6 Hz, 51-C), 117.6 (d, J=24.9 Hz, 52-C), 129.6 (53-C).

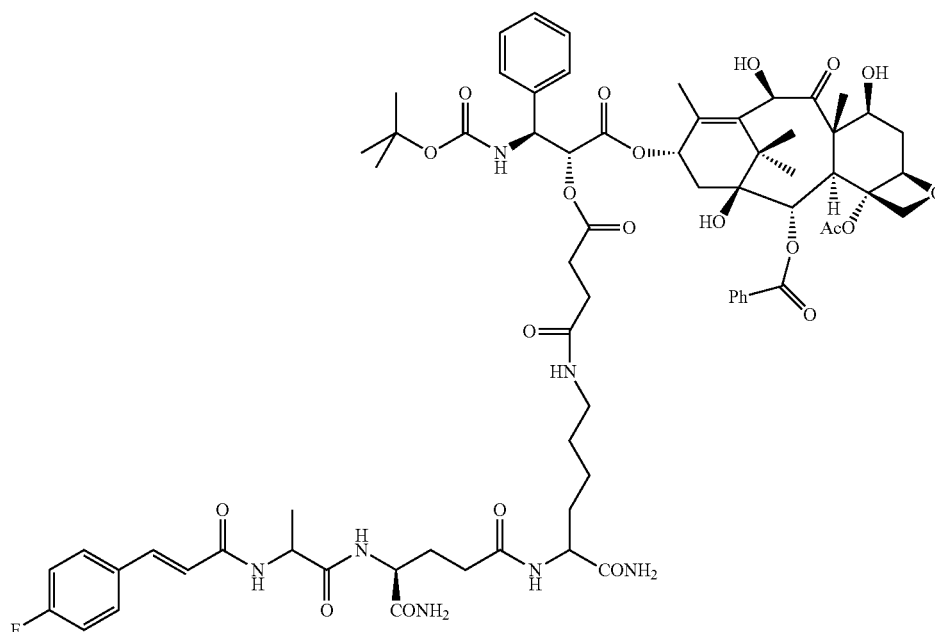
IR: 3316.8 (ν_{OH} and ν_{NH}), 2977.3, 2938.6 (ν_{CH}), 1739.5, 1659.2 ($\nu_{C=O}$), 1533.0, 1490.7 ($\nu_{C=C}$), 1368.3, 1241.6, 1161.7, 1068.6 (δ_{CH}), 982.1, 858.0, 708.6 (δ_{CH}).

ESI-MS: 1416.52 [M+H]⁺, 1438.42 [M+Na]⁺.

HR-MS(TOF): 1416.5725 [M+H]⁺, 1438.5523 [M+Na]⁺, C₇₀H₈₇ClFN₇O₂₁.

Example 41

Liquid-Phase Synthesis of Conjugate MDC 406



90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 49.2 mg (1.0 eq) of muramyl dipeptide analogue MDA-206 was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7~8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 125.6 mg solid product was obtained through lyophilization. Yield 91%, m.p.=162~163° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.41 (1H, br.s, 1-OH), 5.42 (1H, d, J=7.0 Hz, 2-H), 3.65 (1H, d, J=7.0 Hz, 3-H), 4.90 (1H, m, 5-H), 1.66 (1H, m, 6-H_a), 2.25 (1H, m, 6-H_b), 4.03 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.80 (1H, t, J=8.5 Hz, 13-H), 1.64 (1H, m, 14-H_a), 1.82 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 0.99 (3H, s, 17-H), 1.68 (3H, s, 18-H), 1.50 (3H, s, 19-H),

84

3.99 (1H, m, 20-H_a), 4.01 (1H, m, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 5.09 (1H, m, 2'-H), 5.09 (1H, m, 3'-H), 7.86 (1H, m, 3'-NH), 7.30 (2H, m, ph-o-H), 7.35 (2H, m, ph-m-H), 7.16 (1H, t, J=7.0 Hz, ph-p-H), 7.99 (2H, d, J=7.5 Hz, OBz-o-H), 7.65 (2H, m, OBz-m-H), 7.71 (1H, m, OBz-p-H), 1.36 (9H, s, —C(CH₃)₃), 2.55 (2H, m, 22-H), 2.34 (2H, m, 23-H), 7.83 (1H, m, 25-H), 3.01 (2H, br.s, 26-H), 1.21 (2H, m, 27-H), 1.27 (2H, m, 28-H), 1.52 (1H, m, 29-H_a), 1.64 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.97 (1H, s, 32-H_a), 7.31 (1H, s, 32-H_b), 7.86 (1H, m, 33-H), 2.17 (2H, m, 35-H), 1.79 (1H, m, 36-H_a), 2.00 (1H, m, 36-H_b), 4.15 (1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.31 (1H, s, 39-H_b), 8.22 (1H, d, J=8.0 Hz, 40-H), 4.38 (1H, m, 42-H), 1.26 (3H, m, 43-H), 8.35 (1H, d, J=8.0 Hz, 44-H), 6.71 (1H, d, J=16.0 Hz, 46-H), 7.38 (1H, d, J=16.0 Hz, 47-H), 7.87 (2H, m, 49 and 53-H), 7.38 (2H, m, 50 and 52-H).

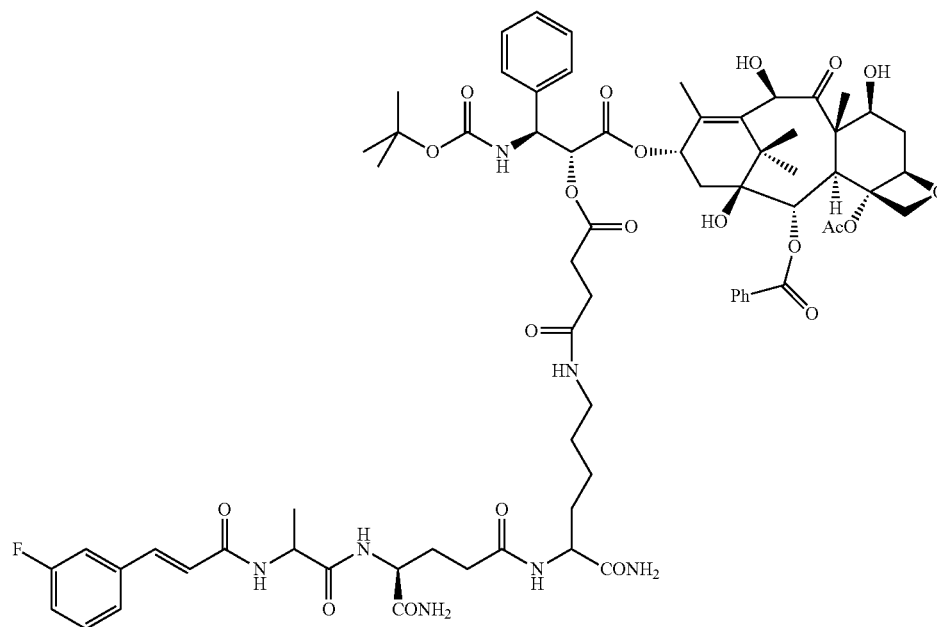
¹³C-NMR (125 MHz, DMSO-d₆): 77.3 (1-C), 75.3 (2-C), 46.4 (3-C), 80.7 (4-C), 84.2 (5-C), 36.9 (6-C), 71.2 (7-C), 57.4 (8-C), 209.8 (9-C), 74.2 (10-C), 136.5 (11-C), 137.2 (12-C), 71.6 (13-C), 35.1 (14-C), 43.3 (15-C), 26.9 (16-C), 21.2 (17-C), 14.1 (18-C), 10.3 (19-C), 75.9 (20-C), 165.8 (2-OCO), 170.0, 22.9 (4-OCOCH₃), 169.4 (1'-C), 75.5 (2'-C), 55.5 (3'-C), 155.7 (3'-NHCO), 79.0, 28.5 (—C(CH₃)₃), 137.9 (ph-q-C), 127.9 (ph-o-C), 129.2 (ph-m-C), 128.5 (ph-p-C), 130.5 (OBz-q-C), 130.1 (OBz-o-C), 129.3 (OBz-m-C), 133.6 (OBz-p-C), 172.3 (21-C), 29.3 (22-C), 30.0 (23-C), 170.5 (24-C), 38.7 (26-C), 29.2 (27-C), 23.4 (28-C), 32.1 (29-C), 52.6 (30-C), 174.4 (31-C), 172.0 (34-C), 32.2 (35-C), 28.2 (36-C), 52.8 (37-C), 173.7 (38-C), 172.8 (41-C), 49.3 (42-C), 18.6 (43-C), 165.3 (45-C), 122.3 (46-C), 137.9 (47-C), 133.9 (48-C), 131.9 (m, 49 and 53-C), 116.4 (d, J=21.8 Hz, 50 and 52-C), 163.2 (d, J=245.3 Hz, 51-C).

IR: 3318.8 (ν_{OH} and ν_{NH}), 2977.6, 2938.0 (ν_{CH}), 1659.3 ($\nu_{C=O}$), 1535.1, 1511.9, 1452.6 ($\nu_{C=C}$), 1368.5, 1246.7, 1160.7, 1069.1 (δ_{CH}), 983.0, 832.9, 708.1 (δ_{CH}).

ESI-MS: 1382.00 [M+H]⁺, 1404.60 [M+Na]⁺.

HR-MS(TOF): 1382.6064 [M+H]⁺, 1404.5900 [M+Na]⁺, C₇₀H₈₈FN₇O₂₁.

Liquid-Phase Synthesis of Conjugate MDC 407



90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 49.2 mg (1.0 eq) of muramyl dipeptide analogue MDA-207 was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7~8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 117.4 mg solid product was obtained through lyophilization. Yield 85%, m.p.=174~175° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.43 (1H, br.s, 1-OH), 5.41 (1H, d, J=7.5 Hz, 2-H), 3.65 (1H, d, J=7.5 Hz, 3-H), 4.91 (1H, m, 5-H), 1.66 (1H, m, 6-H_a), 2.25 (1H, m, 6-H_b), 4.05 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.80 (1H, m, 13-H), 1.64 (1H, m, 14-H_a), 1.82 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 102 (3H, s, 17-H), 1.68 (3H, s, 18-H), 1.51 (3H, s, 19-H), 4.02 (1H, m, 20-H_a), 4.05 (1H, d, J=9.0 Hz, 20-H_b), 2.22 (3H, s, 4-OCOCH₃), 5.09 (1H, m, 2'-H), 5.09 (1H, m, 3'-H), 7.86 (1H, m, 3'-NH), 7.31 (2H, m, ph-o-H), 7.37 (2H, d, J=7.5 Hz, ph-m-H), 7.17 (1H, m, ph-p-H), 7.99 (2H, d, J=7.5 Hz, OBz-o-H), 7.65 (2H, t, J=7.5 Hz, OBz-m-H), 7.74 (1H, m, OBz-p-H), 1.39 (9H, s, —C(CH₃)₃), 2.62 (2H, m, 22-H), 2.36 (2H, m, 23-H), 7.83 (1H, m, 25-H), 3.00 (2H, br.s, 26-H), 1.25 (2H, m, 27-H), 1.26 (2H, m, 28-H), 1.57 (1H, m, 29-H_a), 1.64 (1H, m, 29-H_b), 4.11 (1H, m, 30-H), 6.97 (1H, s, 32-H_a), 7.31 (1H,

s, 32-H_b), 7.92 (1H, m, 33-H), 2.16 (2H, m, 35-H), 1.74 (1H, m, 36-H_a), 2.00 (1H, m, 36-H_b), 4.14 (1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.31 (1H, s, 39-H_b), 8.23 (1H, d, J=8.5 Hz, 40-H), 4.39 (1H, m, 42-H), 1.28 (3H, m, 43-H), 8.37 (1H, d, J=6.5 Hz, 44-H), 6.81 (1H, d, J=16.5 Hz, 46-H), 7.38 (1H, d, J=16.5 Hz, 47-H), 7.37 (1H, m, 49-H), 7.22 (1H, m, 51-H), 7.47 (1H, m, 52-H), 7.41 (1H, m, 53-H).

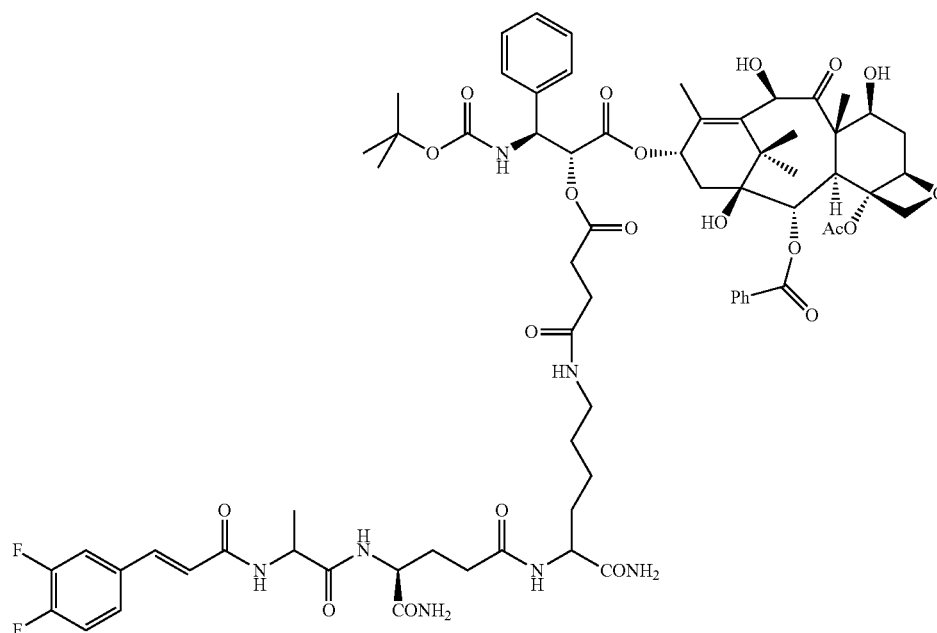
¹³C-NMR (125 MHz, DMSO-d₆): 77.3 (1-C), 75.3 (2-C), 46.4 (3-C), 80.8 (4-C), 84.2 (5-C), 36.9 (6-C), 71.2 (7-C), 57.4 (8-C), 209.8 (9-C), 74.2 (10-C), 136.5 (11-C), 137.3 (12-C), 71.6 (13-C), 35.2 (14-C), 43.3 (15-C), 26.9 (16-C), 21.2 (17-C), 14.1 (18-C), 10.3 (19-C), 75.9 (20-C), 165.1 (2-OCO), 170.0, 22.9 (4-OCOCH₃), 169.4 (1'-C), 75.5 (2'-C), 55.6 (3'-C), 155.7 (3'-NHCO), 78.9, 28.6 (—C(CH₃)₃), 137.9 (ph-q-C), 127.9 (ph-o-C), 129.2 (ph-m-C), 128.5 (ph-p-C), 130.5 (OBz-q-C), 130.1 (OBz-o-C), 129.3 (OBz-m-C), 133.9 (OBz-p-C), 172.3 (21-C), 29.3 (22-C), 30.1 (23-C), 170.6 (24-C), 38.7 (26-C), 29.3 (27-C), 23.4 (28-C), 32.1 (29-C), 52.6 (30-C), 174.4 (31-C), 172.0 (34-C), 32.2 (35-C), 28.2 (36-C), 52.8 (37-C), 173.7 (38-C), 172.8 (41-C), 49.3 (42-C), 18.6 (43-C), 165.8 (45-C), 124.0 (46-C), 138.0 (47-C), 133.9 (48-C), 114.4 (d, J=21.4 Hz, 49-C), 162.9 (d, J=242.4 Hz, 50-C), 116.7 (d, J=21.3 Hz, 51-C), 131.4 (d, J=8.5 Hz, 52-C), 124.1 (d, J=2.5 Hz, 53-C).

IR: 3301.8 (ν_{OH} and ν_{NH}), 2969.9, 2932.2 (ν_{—CH}), 1656.3 (ν_{C=O}), 1529.6, 1449.4 (ν_{C=C}), 1367.3, 1245.0, 1159.9, 1069.2 (δ_{—CH}), 981.7, 783.2, 707.7 (δ_{—CH}).

ESI-MS: 1382.83 [M+H]⁺, 1404.64 [M+Na]⁺.

HR-MS(TOF): 1382.6118 [M+H]⁺, 1404.5942 [M+Na]⁺, C₇₀H₈₈FN₇O₂₁.

Liquid-Phase Synthesis of Conjugate MDC 408



90.7 mg (1.0 eq) docetaxel-2'-O-succinic acid monoester, 11.5 mg (1.0 eq) HOSu and 19.2 mg (1.0 eq) EDC.HCl were dissolved in DMSO, and the mixture was stirred at r.t for 4 hours. 51 mg (1.0 eq) of muramyl dipeptide analogue MDA-208 was sparingly added to the mixture in a few portions, and the pH of the mixture was adjusted to 7~8 with N-methyl morphine. The mixture was continued to stir for 4 hours. After the completion of the reaction, a plenty of water was added to the mixture, and white solids precipitated. The mixture was filtered, and the crude product was obtained. The crude product was purified by ODS column chromatography, and 117.5 mg solid product was obtained through lypophilization. Yield 84%, m.p.=172~173° C.

¹H-NMR (500 MHz, DMSO-d₆): 4.43 (1H, br.s, 1-OH), 5.41 (1H, d, J=7.0 Hz, 2-H), 3.64 (1H, d, J=7.5 Hz, 3-H), 4.90 (1H, m, 5-H), 1.66 (1H, m, 6-H_a), 2.25 (1H, m, 6-H_b), 4.02 (1H, m, 7-H), 5.09 (1H, s, 10-H), 5.80 (1H, m, 13-H), 1.64 (1H, m, 14-H_a), 1.82 (1H, m, 14-H_b), 0.99 (3H, s, 16-H), 102 (3H, s, 17-H), 1.70 (3H, s, 18-H), 1.51 (3H, s, 19-H), 4.02 (1H, m, 20-H_a), 4.05 (1H, m, 20-H_b), 2.25 (3H, s, 4-OCOCH₃), 5.09 (1H, m, 2'-H), 5.09 (1H, m, 3'-H), 7.87 (1H, m, 3'-NH), 7.31 (2H, m, ph-o-H), 7.37 (2H, d, J=7.5 Hz, ph-m-H), 7.19 (1H, m, ph-p-H), 7.99 (2H, d, J=7.0 Hz, OBz-o-H), 7.66 (2H, t, J=7.0 Hz, OBz-m-H), 7.73 (1H, m, OBz-p-H), 1.39 (9H, s, —C(CH₃)₃), 2.62 (2H, m, 22-H), 2.39 (2H, m, 23-H), 7.83 (1H, m, 25-H), 3.01 (2H, br.s, 26-H), 1.25 (2H, m, 27-H), 1.26 (2H, m, 28-H), 1.64 (1H, m, 29-H_a), 1.67 (1H, m, 29-H_b), 4.13 (1H, m, 30-H), 6.97 (1H, s, 32-H_a), 7.31 (1H, s, 32-H_b), 7.92 (1H, m, 33-H), 2.16 (2H, m, 35-H), 1.78 (1H, m, 36-H_a), 2.00 (1H, m, 36-H_b), 4.14 (1H, m, 37-H), 7.11 (1H, s, 39-H_a), 7.31 (1H, s, 39-H_b), 8.22 (1H, d, J=8.0 Hz, 40-H), 4.40 (1H, m, 42-H), 1.28 (3H, m, 43-H), 8.34 (1H, d, J=7.0 Hz, 44-H), 6.74 (1H, d, J=15.5 Hz, 46-H), 7.38 (1H, d, J=15.5 Hz, 47-H), 7.68 (1H, m, 50-H), 7.45 (1H, m, 52-H), 7.49 (1H, m, 53-H).

¹³C-NMR (125 MHz, DMSO-d₆): 77.3 (1-C), 75.3 (2-C), 46.4 (3-C), 80.8 (4-C), 84.2 (5-C), 37.0 (6-C), 71.2 (7-C), 57.4 (8-C), 209.8 (9-C), 74.2 (10-C), 136.5 (11-C), 137.3 (12-C), 71.6 (13-C), 35.2 (14-C), 43.3 (15-C), 26.9 (16-C), 21.2 (17-C), 14.1 (18-C), 10.3 (19-C), 75.9 (20-C), 165.0 (2-OCO), 170.0, 22.9 (4-OCOCH₃), 169.4 (1'-C), 75.5 (2'-C), 55.6 (3'-C), 155.7 (3'-NHCO), 79.0, 28.6 (—C(CH₃)₃), 138.0(ph-q-C), 127.9 (ph-o-C), 129.1 (ph-m-C), 128.5 (ph-p-C), 130.5 (OBz-q-C), 130.0 (OBz-o-C), 129.1 (OBz-m-C), 133.9 (OBz-p-C), 172.3 (21-C), 29.3 (22-C), 30.1 (23-C), 170.4 (24-C), 38.7 (26-C), 29.3 (27-C), 23.4 (28-C), 32.1 (29-C), 52.6 (30-C), 174.4 (31-C), 172.0 (34-C), 32.2 (35-C), 28.2 (36-C), 52.8 (37-C), 173.7 (38-C), 172.7 (41-C), 49.3 (42-C), 18.7 (43-C), 165.7 (45-C), 123.8 (s, 46-C), 137.3 (s, 47-C), 133.3 (m, 48-C), 118.6 (d, J=17.1 Hz, 49-C), 151.2 (m, 50-C), 149.3 (dd, J=34.8 and 13.0 Hz, 51-C), 116.7 (d, J=17.6 Hz, 52-C), 125.1 (m, 53-C).

IR: 3308.5 (ν_{OH} and ν_{NH}), 2977.6, 2936.9 (ν_{CH}), 1659.6 (ν_{C=O}), 1517.9, 1452.4 (ν_{C=C}), 1368.3, 1274.8, 1247.4, 1161.3 (δ_{CH}), 981.7, 775.8, 707.9 (δ_{CH}).

ESI-MS: 1400.82 [M+H]⁺, 1422.63 [M+Na]⁺.

HR-MS(ToF): 1400.6014 [M+H]⁺, 1422.5825 [M+Na]⁺, C₇₀H₈₇F₂N₇O₂₁.

Biological Example

Activity Test In Vitro Part

Example 44

In the invention, six compounds, MTC-220, MTC-302, MTC-213, MTC-219, MTC-233 and MDC-400 were sent to the U.S. National Cancer Institute (NCI) for screening their antitumor activity in vitro. The experimental results show that, the 50% growth inhibition (GI₅₀) activity of those conjugates in 60 human tumor cell lines was in the same magni-

tude range as paclitaxel, and the 50% lethal concentration (LC_{50}) were more than 10 μ M. The experimental results refer to FIGS. 1-12.

In the invention, the compounds, MTC-301, MTC-302, MTC-303, MTC-304, MTC-305, MTC-306, MTC-307, MDC-308, MDC-403, MDC-404, MDC-405, MDC-406, MDC-407 and MDC-408 were screened their antitumor activity in 10 human tumor cell lines. The 50% growth inhibition (GI_{50}) activity of those conjugated compounds was in the same magnitude range as paclitaxel or docetaxel. The experimental results refer to FIGS. 13-16.

Biological Evaluation In Vivo

Example 45

The Tumor Growth Inhibition Activity of MTC-220 in Nude Mice Xenograft Models Using Human Breast Cancer Line MDA-MB-231

Experiment Materials and Test Animals:

1. MTC-220, a colorless and clear liquid had the concentration of 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, was repackaged in a sterile condition, and can be used directly, stored at 4° C. Drug administration dose were set as: 10 mg/kg, 15 mg/kg, and 20 mg/kg, drug administration volume was 0.2 mL/20 g.
2. Paclitaxel Injection, the products of Beijing Union Pharmaceutical Factory, Approval Number: H10980069, product lot number: 080704, specifications 5 mL: 30 mg.
3. Taxol+MDA [Peptide MDA(P) 0.54 mg/mL (0.001M)+ Taxol (T) 0.9 mg/mL (0.001M)], were prepared by the commission, can be directly used after the repackaging in a sterile condition, stored at 4° C.
4. MDA [Peptide (P) 0.54 mg/mL (0.001M), Example 10], a colorless and clear liquid, was prepared by the commission, can be directly used after the repackaging in a sterile condition, stored at 4° C. Tumor lines: Highly metastatic human breast cancer line MDA-MB-231 were implanted in nude mice, and the tumor-bearing mice were obtained from Crown Bioscience Co. Ltd. (Beijing), and were cultured and preserved by our laboratory.

Animals: BALB/c nu mice, ♀, 4~5 weeks old, were obtained from the Institute of laboratory animal, Chinese Academy of Medical Science. Certificate NO. SCXK (Beijing) 2005-0013.

Feeding facilities: Experimental Animal Center, Chinese Academy of Medical Sciences, SPF level Animal Lab, Certificate NO. SYSK (Beijing) 2004-0001.

Experiment Methods:

The tumor-bearing mice with good tumor growth and good general physical condition were selected and sacrificed. Tumor was isolated in a sterile condition and cut into fragments (diameter for about 2-3 mm) by surgical knife. The fragments were then hypodermically inoculated in posterior axillary of nude mice by means of a trocar. The tumor was grown normally. The mice were divided into groups and administrated drug after 11 days. The length and width of tumor were measured using vernier calipers, and divided into groups by the tumor volume.

The mice were divided into eight groups, each group had 6-8 mice. The groups contained Negative control. Paclitaxel group, were injected paclitaxel injection in dose 24 mg/kg intermittently, three MTC groups, were administrated with MTC-220 in a dose of 10 mg/kg, 15 mg/kg and 20 mg/kg respectively, MDA group; and Taxol+MDA group. The tumors sizes of the above 7 groups mice were similar with the

average volume of about 140 mm³. Mice with relatively larger tumor volumes than usual (with an average volume of 340 mm³) were administrated with MTC-220 in a dose of 30 mg/kg (MTC-220 30 mg/kg group). After grouping, all mice were administrated with drug by intraperitoneal injection once a day depends on their body weight.

The day of grouping and administration of drug was defined as D1, the tumors sizes (length and width) and body weights of mice were measured once every three days. The paclitaxel control group was intermittently administered for 4 times, while the MTC-220 with 30 mg/kg group was withdrawn from drug after administration successively for 12 times. Other groups were administered with drugs for 24 times successively. The experiment was completed 24 h after the last administration.

The mice were sacrificed, and tumors were isolated and their weight was measured, and the inhibition rate of tumor growth by drugs were calculated. Statistical significance of the tumor weight, tumor volume and RTV level were evaluated by t-test. Calculation methods and formula were omitted.

Anti-tumor activities were evaluated by Tumor Relative proliferation Rate T/C (%)

Therapeutic effect evaluation standard: T/C (%)>40, was judged as invalid;

T/C (%)≤40, and through statistical evaluation P<0.05, was judged as invalid valid.

Experiment Results:

During the observation of the experiment, the body weight of mice in negative control group gradually increased. The average body weight increased by 3.5 g compared to the beginning of the division. Paclitaxel control group was administrated intermittently, the body weight maintained in the tolerated range of toxic and side effects. The MTC-220 30 mg/kg dose group was administrated 12 times successively in 12 days, and the body weight of mice maintained essentially the same as the that at the beginning of the grouping, but the body weight gradually increased after withdrawal of drug, and at the end of the experiment the body weight increased by 2.6 g compared to the beginning of the grouping. The increase of body weight in MTC-220 30 mg/kg doses group was the same as MTC-220 15 mg/kg dose group which was treated for 24 times (the latter group body weight was increased by 2.7 g), the two groups had similar total administration dose. While the MTC-220 20 mg/kg dose group was administrated successively for 24 days, the body weight of this group increased by 1.9 g, less than the body weight of negative group. The body weight of T (0.9 mg/mL)+P (0.54 mg/mL) group under administration successively was close to the body weight of Paclitaxel group in early stage, but the toxic and side effects appeared gradually during the continued administration, which included abdominal distention, less movement, weight loss, etc. At the twentieth day, 2/3 mice of this group had been died.

The mice tumor growth curve indicated, the tumor growth rate in MDA liquid [P (0.54 mg/mL)] administered group was slower than the tumor growth rate of the negative control group, and the tumor relative proliferation rate (T/C) was 83.5%. The tumor growth was significantly related to the administration dose of MTC-220 10 mg/kg, 15 mg/kg and 20 mg/kg. At the end of experiment, the tumor growth inhibition rate of the three groups were 37.3%, 57.4% and 72.2%, respectively, and tumor relative proliferation rate were 70.0%, 39.5% and 29.4% respectively, wherein the MTC-220 15 mg/kg group and MTC-220 20 mg/kg group were judged as valid.

MTC-220 30 mg/kg group which were administrated successively for 12 times, the total dose was the same as the MTC

15 mg/kg group which were administrated successively for 24 times. Even though the tumor volume of MTC-220 30 mg/kg group was a little bigger at the beginning of the experiment, it became smaller gradually during the administration. The growth rate was also quite slow after withdrawal of drug. At the end of experiment, the tumor growth inhibition activity of MTC-220 30 mg/kg group increased significantly (MTC-220 15 mg/kg group was 57.4%, MTC-220 30 mg/kg group was >87%), and the tumor relative proliferation rate (T/C) decreased significantly (MTC-220 15 mg/kg group was 37.5%, MTC-220 30 mg/kg group was >6.16%). Compared MTC-220 30 mg/kg dose group, which administered successively for 12 times, with MTC-220 20 mg/kg dose group, which was administered successively for 24 times, the amount administered in MTC-220 30 mg/kg group was smaller, but the inhibition rate of MTC-220 30 mg/kg group was higher, the tumor relative proliferation rate (TIC) of MTC-220 30 mg/kg group was also decreased significantly, and the mice physical conditions in MTC-220 30 mg/kg group were better. All above indicated that if the tumor bear-

ing mice were administered with suitable dose, not only the tumor growth can be controlled, but also less dose and shorter treatment is needed, and further the toxic and side effects are decreased.

Experiment conclusion: The inhibition of human breast cancer MDA-MB-231 in tumor bearing nude mice was significant after the mice were injected intraperitoneally with MTC-220 10 mg/kg, 15 mg/kg and 20 mg/kg successively. The growth of MDA-MB-231 tumor line was inhibited significantly, and the inhibition effects were related to the administration dose. The administration effects of 15 mg/kg and 20 mg/kg were judged as valid in this lot experiment.

MTC-220 30 mg/kg group were administrated successively for 12 times, the inhibition of the tumor growth of MDA-MB-231 was significant. The tumor grew slowly after withdrawal of drug, and the physical condition recovered well. The treatment period was shorter, and the effect of tumor inhibition was more significant compared to the MTC-220 15 mg/kg group. The experiment results refer to FIGS. 11-14 and Table 1-2.

TABLE 1

The effect of MTC-220 in MDA-MB-231 xenograft tumor nude mice (1)						
Group	Mice NO.		Body weight (g)		Tumor weight	TGI
	Beginning	End	Beginning	End	(g)	(%)
NC	7	7	19.0 ± 1.14	22.5 ± 1.92	2.84 ± 1.205	
Paclitaxel	8	8	17.7 ± 1.50	19.5 ± 0.94	0.43 ± 0.416***	84.9
24 mg/kg × 4						
MTC-220	6	6	17.4 ± 1.47	20.6 ± 1.64	1.78 ± 1.016	37.3
10 mg/kg × 24						
MTC-220	6	6	17.9 ± 0.88	20.6 ± 0.91	1.21 ± 0.813*	57.4
15 mg/kg × 24						
MTC-220	7	7	17.0 ± 1.11	18.9 ± 1.58	0.79 ± 0.654**	72.2
20 mg/kg × 24						
MTC-220	6	6	17.5 ± 1.09	20.1 ± 0.98	0.37 ± 0.413***	>87.0
30 mg/kg × 12						
Taxol + MDA × 24	6	2	17.4 ± 1.09	19.2 ± 0.05	0.77 ± 0.440	72.9
MDA × 24	6	6	18.5 ± 1.05	21.4 ± 0.90	1.98 ± 0.744	30.3

*P < 0.05, Compared to NC.

**P < 0.01, Compared to NC.

***P < 0.001, Compared to NC.

(TGI, Tumor Growth Inhibition; NC, Negative Control)

TABLE 2

The effect of MTC-220 in MDA-MB-231 xenograft tumor nude mice (2)				
Group	Tumor Volume (mm ³)			T/C
	Beginning	End	RTV	(%)
NC	138 ± 48.4	2388 ± 1073.6	18.03 ± 6.108	
Paclitaxel	133 ± 39.8	422 ± 404.6	3.18 ± 2.735***	17.64
24 mg/kg × 4				
MTC-220	135 ± 70.6	1655 ± 929.4	12.62 ± 5.924	70.00
10 mg/kg × 24				
MTC-220	148 ± 80.5	967 ± 590.4	7.12 ± 4.064**	39.49
15 mg/kg × 24				
MTC-220	133 ± 57.6	642 ± 482.3	4.58 ± 2.456***	25.40
20 mg/kg × 24				
MTC-220	340 ± 58.4	391 ± 480.5	1.11 ± 1.366***	6.16
30 mg/kg × 12				
Taxol + MDA × 24	136 ± 40.7	1093 ± 343.3	11.70 ± 0.299*	64.9
MDA × 24	141 ± 61.1	1898 ± 775.4	15.06 ± 5.292	83.5

*P < 0.05, Compared to NC.

**P < 0.01, Compared to NC.

***P < 0.001, Compared to NC.

(TGI, Tumor Growth Inhibition; NC, Negative Control)

The Growth Inhibition of MTC-220 in Human Lung Cancer H460 Xenograft Tumor Nude Mice

Experiment Materials and Test Animals:

MTC-220: It was prepared by the commission, three concentrations of 1.0 mg % mL, 1.5 mg/mL and 2.0 mg/mL, was colorless and clear liquid, was dispensed in a sterile condition and can be used directly, stored at 4° C.

Paclitaxel Injection: the product of Beijing Union Pharmaceutical Factory, Approval Number: H10980069, product lot number: 080704, specifications 5 mL: 30 mg. Solvent Vehicle: (the physiological saline solution mixture contained 5% DMSO and 5% polyoxyethylene alcohol castor oil (Cremphor EL)), was dispensed in a sterile condition and can be used directly, stored at 4° C.

Tumor lines: Human lung cancer H460 cell lines were obtained from ATCC, and was cultured and preserved in the Lab. Through cell culture in vitro, the tumor was inoculated on nude mice, the tumor grew and passaged for the experiment use.

Animals: BALB/c nude mice, ♀, 4~5 weeks old, were obtained from the Experimental Lab, Chinese Academy of Medical Science, Certificate NO. SCXK (Beijing) 2005-0013.

Feeding facilities: Experimental Animal Center SPF level Animal Lab, Chinese Academy of Medical Sciences, Certificate NO. SYSK (Beijing) 2004-0001.

Experiment Method:

The tumor-bearing mice with good tumor growth and good general physical condition were selected and sacrificed. Tumor was isolated in a sterile condition and cut into fragments (diameter for about 2-3 mm) by surgical knife. The fragments were then hypodermically inoculated in posterior axillary of nude mice by means of a trocar.

After the tumors grew naturally for eight days, the average volume of tumors reaches 130 mm³. The length and width of tumor was measured using vernier calipers, and divided into groups by the tumor volume.

The mice were divided into five groups for observation, each groups had eight mice. The negative control group was administered with solvent vehicle, and the other three dose groups were administered with MTC-220 5 mg/kg, 10 mg/kg, 20 mg/kg, respectively. The positive control group was administered with paclitaxel injection in a dose of 24 mg/kg once every three days. Respective drug was administrated for each group from the grouping day.

The grouping day was defined as D1, the administration of the paclitaxel control group was administered intermittently for 4 times, while MTC-220 groups were administered for 25 times successively. The experiment was terminated 24 hours after the last administration.

During the experimentation, the tumors sizes (length and width) and body weights of mice were measured once every three days. The tumor volume (TV) and relative tumor volume (RTV) were calculated according to the method for references, and the tumor volume growth tendency chart was plotted.

At the end of the experiment, the mice were sacrificed. Tumors were removed and weighed, and the inhibition rate of the tumor growth by drugs was calculated. Statistical significance of the tumor weight, tumor volume and RTV level were evaluated by t-test.

Calculation formula:

$$\text{Tumor growth inhibition}(\%) = \frac{C - T}{C} \times 100\%$$

(C, average tumor weight of control group; T, average tumor weight of administrated group)

Tumor Volume (TV)=length×width²/2.

Relative Tumor Volume (RTV) formula: V_t/V_0

(V_0 is the volume of TV at the beginning of the grouping, and V_t is the volume of TV at measure time)

Anti-tumor activities were evaluated by Tumor Relative proliferation Rate T/C (%)

$$T/C(\%) = \frac{\text{Administrated Group (T) RTV}}{\text{Negative control group (C) RTV}} \times 100\%$$

Therapeutic effect evaluation standard: T/C (%)>40, was judged as invalid;

T/C (%)≤40, and through statistical evaluation P<0.05, was judged as valid.

Experiment Results:

The observed results demonstrated that, during the 25 days, the body weight of negative control group gradually increased, and general status had no change. H460 tumor grew faster, compared with the tumor volume at the beginning of the grouping, the average of negative control relative tumor volume was 33.3 at the end of the experiment.

Positive control group which was administered with paclitaxel in dose of 24 mg/kg twice a day, indicated its inhibition of the growth of H460 tumor. The tumor growth inhibition rate gradually increased with the increase of administration times. Compared with negative control group the tumor growth inhibition rate was 65% after the fourth administration. The therapeutic effects maintained for one week after withdrawal of drug, and decreased gradually thereafter. At the end of the experiment, the statistics results indicated the inhibition rate of tumor weight was 61%, and the tumor relative proliferation rate (T/C) was 35.6%. The therapeutic effect of the positive control group was better than the negative control group. It was also observed in the experiment that, after the administration with paclitaxel in the dose of 24 mg/kg twice intermittently, the mice started losing weight and the weight lost gradually by 2 compared with the average weight at the beginning of grouping. The body weight started to recover one week after withdrawal drug.

Twenty days before the administration, the mouse weight was essentially the same between the negative control group and two groups which were treated with MTC-200 10 mg/kg and 5 mg/kg, respectively. The body weight of the two treated groups decreased somewhat compared to the negative control group during the continued administration. After 25 days of the successive administration with a dose of MTC-220 5 mg/kg, the growth rate of tumor volume was not significantly different compared to that of the negative control. After 2 weeks of the successive administration with a dose of 10 mg/kg, the measured result of H460 tumor volume was different from that of the negative control. At the end of the experiment, the tumor volume inhibition of 10 mg/kg dose group was 18.8%, and the tumor weight inhibition rate was 17.3%.

After 10 days of treatment with MTC-220 in a dose of 20 mg/kg, the measured result of tumor volume was different from that of the negative control group. Tumor grew slowly during the continued administration, and the inhibition of tumor growth gradually increased. Until the end of experiment, the inhibition of tumor weight was 52.9%, and the tumor relative proliferation Rate (T/C) was 50.1%, it was significant in statistics compared with the negative control group. Experiment results refer to FIGS. 15-16 and table 3-4.

TABLE 3

MTC-220 effects H460 tumor growth inhibition (1)						
Group	Mice NO.		Body weight (g)		Tumor weight	TGI
	Beginning	End	Beginning	End	(g)	(%)
NC	8	8	18.3 ± 0.71	22.6 ± 1.30	2.98 ± 0.626	
MTC-220	8	8	18.0 ± 0.95	21.9 ± 1.10	2.91 ± 0.695	2.15
5 mg/kg × 25						
MTC-220	8	8	18.2 ± 0.70	21.4 ± 1.15	2.46 ± 0.624	17.3
10 mg/kg × 25						
MTC-220	8	6	17.8 ± 1.10	18.9 ± 2.49	1.40 ± 0.466**	52.9
20 mg/kg × 25						
Paclitaxel injection	8	8	18.9 ± 1.28	18.6 ± 1.41	1.16 ± 0.410**	61.0
24 mg/kg × 4						

**P < 0.05, compared to negative group.

(TGI, Tumor Growth Inhibition; NC, Negative Control)

TABLE 4

MTC-220 effects H460 tumor growth inhibition (2)				
Group	Tumor volume (mm ³)			T/C
	Beginning	End	RTV	(%)
Negative control	133 ± 39.1	4032 ± 751.0	33.3 ± 13.21	
MTC-220	125 ± 36.8	3737 ± 591.0	32.0 ± 8.27	96.2
5 mg/kg × 25				
MTC-220	125 ± 43.0	3274 ± 797.0	27.7 ± 6.81	83.1
10 mg/kg × 25				
MTC-220	123 ± 44.6	1963 ± 641.9	16.7 ± 9.93**	50.1
20 mg/kg × 25				
Paclitaxel injection	130 ± 36.7	1583 ± 507.2	11.9 ± 3.16**	35.6
24 mg/kg × 4				

**P < 0.05, compared to negative group

(RTV, Relative Tumor Volume)

Experiment result: Human lung tumor H460 bearing mice were injected intraperitoneally by the successive administration with MTC-220 in dose of 5 mg/kg, 10 mg/kg, 20 mg/kg for 25 days respectively. The MTC sample inhibited the growth of H460 tumor, and the inhibition effects of anti-tumor were related to the drug dosage. At the end of experiment, the inhibition of tumor weight of the 20 mg/kg dose group was 52.9%, relative tumor proliferation rate was 50.1%, they were significantly different in statistics compared with them of the negative group.

Example 47

The Screening Results of MTC-220 in Xenograft Tumor Nude Mice Using the Sensitive Tumor Lines

Experiment Purpose: To test the effect of MTC-220 in xenograft tumor nude mice using breast cancer, lung cancer and ovarian cancer tumor cell lines in vivo. The tumor lines which were significantly sensitive to MTC-220 was screened, and the response of nude mice during the successive administration was observed.

Experiment Animals: BALB/c nu mice were obtained from the institute of Laboratory Animal, Chinese Academy of Medical Science. Certificate NO. SCXK (BeiJing) 2005-0013.

Cell lines: The tumor cell lines were passaged and cultured by our Lab, some of them was obtained from ATCC.

The tumor cell lines included: Human breast cancer MX-1 and MCF-7.

Human ovarian cancer A2780, and clear Human ovarian cell cancer ES-2,

Human lung cancer H1975 and A549.

Experiment Method:

1. The mice were only divided into negative group and MTC-220 administration group.
2. The method was essentially the same as Example 52 and Example 53, which is not described in detail here.
3. The administration dose and treatment progress was determined based from the preliminary experiments, which had solid effects and the shortest treatment period—the dose of 30 mg/kg/day, and the administration duration time of every lot experiment was not more than 12 days.

Experiment results (1): After the administration of MTC-220, the MCF-7 tumor of mice became smaller. At the tenth administration, the tumor volume was very small, then drug was withdrawn and the MCF-7 tumor of mice was under observation. After another week, the tumor of the group disappeared one after another. There was no tumor discovered during the following three weeks of the continued observation. Only the breast cancer MCF-7 tumor grew slowly. Fifty days after inoculation, the tumor volume of negative group was no more than 600 mm. The observation was terminated because the experiment result was clear.

The change in body weight can be found in the Figures, and the drug had certain effect on the body weight, the body weight had a tendency of decrease during the administration. The body weight increased after drug withdrawal, and the change was essentially parallel as negative control group. Experiment results refer to FIGS. 17-18 and Table 5-6.

TABLE 5

The body weight at the beginning and end of the experiment, and the MCF-7 tumor weight at the end of the experiment (1)						
Group	Mice NO.		Body weight (g)		Tumor Weight	TGI
	Beginning	End	Beginning	End	(g)	(%)
NC	6	5	19.4 ± 1.72	22.7 ± 1.21	0.558 ± 0.275	
MTC-220 30 mg/kg × 12	6	6	20.8 ± 1.14	23.3 ± 1.22	0***	100

**P < 0.05

(TGI, Tumor Growth Inhibition; NC, Negative Control)

TABLE 6

The MCF-7 tumor volume at the beginning and end of the experiment (2)				
Group	Tumor volume (mm ³)			T/C
	Beginning	End	RTV	(%)
NC	136 ± 73.1	573 ± 286.4	5.29 ± 1.432	
MTC-220 30 mg/kg × 12	142 ± 73.5	0	0***	0

**P < 0.05;

(RTV, Relative Tumor volume; NC, Negative Control)

Experiment results (2): During the administration of MTC-220, A549 tumor became smaller and smaller, but didn't disappear. One week after withdrawal of drug, the tumor of one mouse disappeared. Within two weeks after withdrawal of drug, the average volume of MTC-220 administration group was maintained at the level at the time of drug withdrawal, it didn't increase.

The change of body weight was shown in the Figures, and the drug had an observable effect on the body weight, the body weight decreased continuously during the administration. The body weight kept decreasing within several days after drug withdrawal, one mouse died one week after drug withdrawal, and the body weight of other mice recovered gradually. Experiment results were shown FIGS. 19-20 and Table 7-8.

TABLE 7

The body weight at the beginning and end of the experiment and the tumor weight of A549 at the end of the experiment (1)						
Group	Mice No.		Body weight (g)		Tumor weight	TGI
	Beginning	End	Beginning	End	(g)	(%)
NC	6	6	24.1 ± 1.90	29.3 ± 1.82	0.31 ± 0.100	
MTC-220 30 mg/kg × 12	6	5	25.2 ± 1.31	26.3 ± 1.51	0.062 ± 0.041***	79.9

**P < 0.05

(TGI, Tumor Growth Inhibition; NC, Negative Control)

TABLE 8

The tumor volume of A549 at the beginning and end of the experiment (2)				
Group	Tumor volume (mm ³)			T/C
	Beginning	End	RTV	(%)
NC	93 ± 29.5	268 ± 100.5	2.87 ± 0.562	
MTC-220 30 mg/kg × 12	95 ± 27.7	74 ± 55.2	0.67 ± 0.411**	23.3**

**P < 0.05;

(RTV, Relative Tumor volume; NC, Negative Control)

Experiment results (3): The MTC-220 administration significantly inhibited H1975 tumor growth of. During the administration, the tumor volume of treated group became smaller and smaller, and then the tumor in some mice disappeared. Experiment results were shown in FIGS. 21-22, and Table 9-10.

TABLE 9

The tumor weight of H1975 at the beginning and end of the experiment (1)						
Group	Mice NO.		Body weight (g)		Tumor weight	TGI
	Beginning	End	Beginning	End	(g)	(%)
NC	7	7	23.8 ± 1.43	27.2 ± 1.23	1.91 ± 0.909	
MTC-220 30 mg/kg × 12	7	5	24.1 ± 1.20	26.6 ± 0.76	0.13 ± 0.103**	93.1

**P < 0.05

(TGI, Tumor Growth Inhibition; NC, Negative Control)

TABLE 10

The experiment beginning and end H1975 tumor volume (2)				
Group	Tumor volume (mm ³)			T/C
	Beginning	End	RTV	(%)
NC	117 ± 60.0	1490 ± 621.2	13.08 ± 2.541	
MTC-220 30 mg/kg × 12	135 ± 50.6	127 ± 106.1	0.66 ± 0.464**	5.0

**P < 0.05;

(TGI, Relative Tumor volume; NC, Negative Control)

The conclusion of screening MTC-220 on tumor:

MTC-220 was applied on human breast cancer, lung cancer, ovarian cancer of xenograft tumor nude mice, the screening results of preliminary experiments indicated, mice injected intraperitoneally with MTC-220 in the dose of 30 mg/kg for 10~12 times demonstrated that the MTC samples had inhibition effects on the growth of the selected tumor with different degrees in the screening experiment.

It was observed that from the experiment, the inhibition of MTC-220 on the growth of breast cancer MX-1 was weak, the inhibition of MTC-220 on ovarian cancer A2780 and ES-2 tumor was at certain degree, but didn't attain the valid standard. MTC-220 demonstrated significant inhibition effects on breast cancer MCF-7, lung cancer A549 and H1975 tumor. The observed result indicated that, in MTC-220 sensitive tumor lines, the tumor volume of bearing mice became smaller during administration, after drug withdrawal the tumor volume kept decreasing, and the tumors in some mice disappeared. At the end of the experiment, the inhibition of A549 and H1975 tumor growth were above 80%, their tumor relative proliferation rates were below 30%, which were significantly statistically different compared with the negative control group. The MTC-220 inhibited MCF-7 tumor growth significantly, and the tumor of treated mice group disappeared after successive administration for 10 times.

Conclusion: MTC-220 inhibited breast cancer and lung cancer significantly, it is most sensitive to the tumor lines of MDA-MB-231, MCF-7, H460, H1975 and A549.

Example 48

Anti-Natural Metastasis Effect of MTC-220 on Breast Cancer in Mice

Mice breast cancer cell line (4T1, ATCC CRL2539) was a generous gift from Prof. Wei Liang of the Institute of biophysics, Chinese Academy of Sciences. The cell was cultured in the 1640 medium (Gibco) containing 10% fetal bovine serum (FBS), 1% glutamine and 1% penicillin.

4T1 cells in logarithmic phase were collected and the concentration was adjusted to 2×10^6 /mL. 4T1 tumors were introduced in female BALB/c mice by injecting subcutaneously

into the fourth fat pad area of the right abdominal mammary gland with the dose of 2×10^5 /0.1 mL. Five days after the implantation of 4T1 tumor cells, the mice were divided into five groups randomly, each group had eight mice, and the mice were respectively received intraperitoneal administration of paclitaxel (3 mg/kg), MTC-220 (2.5 mg/kg, 5 mg/kg, 10 mg/kg) or control vehicle once daily. From the 9th day after implantation, tumor growth was measured every 2 days with vernier calipers for determining the long diameter and short diameter of tumor. Tumor volume was calculated by the formula $(\frac{1}{2}) \times \text{long diameter} \times \text{short diameter}^2$. Drug was withdrawn on the twenty-eight days after the implantation, all mice were then sacrificed and the body weight were measured. The tumors, spleen and lung were removed and weighed. The lungs were fixed in Bouin's fixative for 24 h. The numbers of lung metastasis nodule were counted, the statistics was evaluated using Mann-Whitney U test.

The results indicated that, MTC-220 significantly decreased the lung metastasis nodule numbers of 4T1 mice with statistical significance ($p < 0.01$) compared to vehicle control group, and the result depended on the administration dosage. There was no significant improvement of lung metastasis nodule in the Taxol group. MTC-220 and Taxol both significantly inhibited the growth of tumor compared to vehicle control group. During the observation of the experiment, there was no toxic and side effects of MTC-220 observed. Experiment results were shown in FIGS. 23-25 and Table 11.

TABLE 11

MTC-220 Anti-natural metastasis activities of mice breast cancer			
Group	Tumor weight (g)	Lung weight (mg)	Lung metastasis nodule counts
Vehicle	1.08 ± 0.3	163 ± 11	39 ± 13
TAXOL (3 mg/kg)	0.80* ± 0.2	190 ± 49	41.2 ± 9
MTC-220 (2.5 mg/kg)	0.84* ± 0.2	153 ± 18	18.1**ΔΔV ^Δ ± 3
MTC-220 (5.0 mg/kg)	0.77* ± 0.2	160 ± 15	13.3**ΔΔV ^Δ ± 5
MTC-220 (10 mg/kg)	0.71** ± 0.2	147*ΔV ± 17	10.6**ΔΔV ^Δ ± 3

Compared to vehicle control group:

**P < 0.01,

*P < 0.05;

Compared to Taxol group:

ΔΔP < 0.01,

ΔP < 0.05;

Example 49

Anti-Natural Metastasis Effect of MTC-220 on Lung Cancer in Mice

C57Bl/6 mice with lewis lung cancer were sacrificed and the tumor was removed. The tumor cell suspension (5×10^6

101

cell/mL) was prepared in a sterile condition. The suspension (0.2 mL/mice, 1×10^6 tumor cell) was inoculated subcutaneously into the axillary of 24 C57Bl/6 mice. Three days after the implantation, the mice were divided into three groups randomly, each group had eight mice, and the mice separately received intraperitoneally administration of paclitaxel (6 mg/kg), MTC-220 (10 mg/kg), or control vehicle once daily. From the 7th day after the implantation, the long diameter and short diameter of tumor was measured every 2 days. Tumor volume was calculated by the formula $(\frac{1}{2}) \times \text{long diameter} \times \text{short diameter}^2$. Drug was withdrawn on the eighteenth day after the implantation. All mice were then sacrificed and the body weight was measured. The tumors, spleen and lung were removed and weighed. The lungs were fixed in Bouin's fixative for 24 h. The numbers of lung metastasis nodule were counted, and the statistics was evaluated by Mann-Whitney U test.

The results indicated that, MTC-220 significantly decreased the lung metastasis nodule number of LLC mice with statistical significance ($p < 0.05$) compared to vehicle control group. There was no significant improvement of lung metastasis nodule in the Taxol group. MTC-220 and Taxol both significantly inhibited the growth of tumor compared to vehicle control group. During the observation of the experiment, there was no toxic and side effect by MTC-220, and the body weight of mice increased gradually. Experiment results were shown in FIGS. 26-28 and Table 12.

TABLE 12

Anti-natural metastasis activities of MTC-220 in Lewis lung cancer mice			
Group	Tumor weight (g)	Lung weight (mg)	Lung metastasis nodule counts
Vehicle	5.75 \pm 1.6	205 \pm 121	31.4 \pm 11
TAXOL (6 mg/kg)	4.21* \pm 1.1	161 \pm 27	24.9 \pm 9
MTC-220 10 mg/kg	3.84* \pm 1.4	152 \pm 37	16.5* ^{AV} \pm 9

Compared to vehicle control group:

**P < 0.01,

*P < 0.05;

Compared to Taxol group:

^{AA}P < 0.01,

^AP < 0.05.

Example 50

Anti-Artificial Metastasis of MTC-220 on Lewis Lung Cancer in Mice

C57Bl/6 mice with Lewis lung cancer were sacrificed and the tumor was removed. The tumor cell suspension (5×10^6 cell/mL) was prepared in a sterile condition. The suspension (0.2 mL/mice, 3×10^5 tumor cell) was inoculated intravenously into the tails of fifty C57Bl/6 mice. Two days after the implantation, the mice were divided into five groups randomly, each group had ten mice, and the mice separately received intraperitoneally administration of paclitaxel (3 mg/kg), MTC-220 (2.5 mg/kg, 5 mg/kg or 10 mg/kg), or control vehicle. Drug was withdrawn on the twenty-eighth day after the successive administration, all mice were then sacrificed and the body weight was measured. The tumors, spleen and lung were removed and weighed. The lungs were fixed in Bouin's fixative for 24 h. The number of lung metastasis nodule was counted, and the statistics was evaluated by Mann-Whitney U test.

102

The results indicated that, MTC-220 significantly decreased the lung metastasis nodule number of LLC mice with statistical significance compared to vehicle control group, and the result depended on the administration dosage. There was no significant improvement of lung metastasis nodule in the Taxol group. Experiment results were shown in FIG. 29 and Table 13.

TABLE 13

Antitartificial metastasis activities of MTC-220 on Lewis lung cancer in mice			
Group	Body weight (g)	Lung weight (mg)	Lung metastasis nodule counts
Vehicle	19.3 \pm 1.3	397 \pm 301	35.0 \pm 21
TAXOL (3 mg/kg)	17.1* \pm 2.8	497 \pm 491	38.5 \pm 28
MTC-220 (2.5 mg/kg)	19.0 \pm 1.9	334 \pm 217	16.4* \pm 7
MTC-220 (5 mg/kg)	18.4 \pm 2.3	492 \pm 353	15.0* \pm 7
MTC-220 (10 mg/kg)	17.4** \pm 1.5	393 \pm 326	11.8** \pm 6.8

Compared to vehicle control group:

**P < 0.01,

*P < 0.05

Example 51

The Toxicity Test of MTC-220 by Single Dose

Experiment Methods:

In light of the publication "technical guidelines for cytotoxic anticancer drugs in non-clinical studies" and "technical guidelines for studies on chemical drugs with acute toxicities" by State Food and Drug Administration, the toxicity study on MTC-220 was conducted at maximal administration dosage in the ICR mice with a single dose was intravenously injected.

Experiment Results:

After the intravenous injection of MTC-220 in a dose of 112.5 mg·kg⁻¹ the voluntary activities of mice in administered group were reduced, some mice showed jumping symptoms, which then recovered about 10 min later. There was no unusual phenomenon in the Vehicle group (Epoxidized castor oil:DMSO:Normal Saline=5:5:90, volume ratio) and Control group. After continued observation for 14 days, the animal behavior, voluntary activities and physical sign of each group were normal, and no death occurred.

The body weight of each administered group and vehicle group was not significantly different compared with that of the control group. Anatomical examination results: animal heart, liver, spleen, lung, kidney, gastrointestinal and other various organs showed no sign of abnormal changes.

Experimental Results:

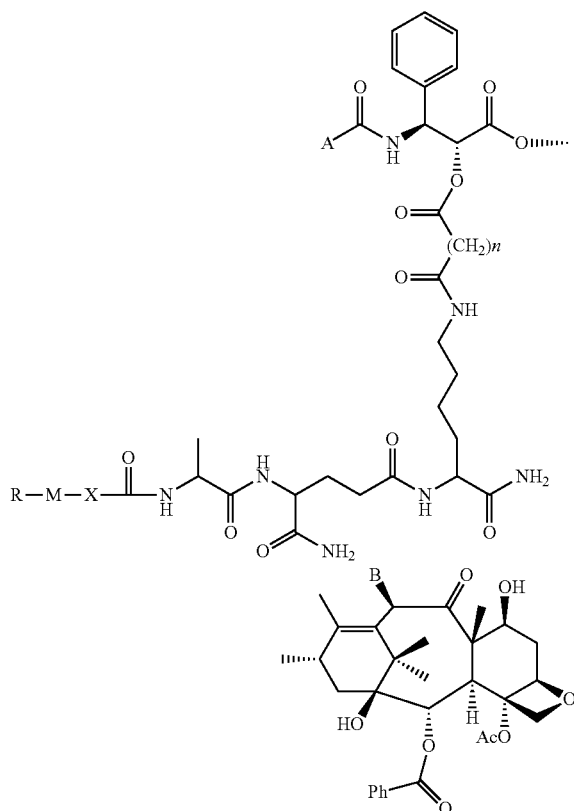
After the intravenous injection to ICR mice tail with MTC-220 in a single dose of 112.5 mg·kg⁻¹, there was no significant symptoms of toxicity or death. It was thought that the MTD of MTC-220 by intravenous injection into the tested ICR mice was higher than its maximum administration dose (112.5 mg·kg⁻¹).

Pharmacology experiment results above, as well as single-dose toxicity test result showed that the design concept of the conjugate of taxane anti-tumor agent and Muramyl Dipeptide Analogue was right. It was a series of safe and new compounds, which can be developed as new drugs with the dual anti-tumor and anti metastasis functions.

103

What is claimed is:

1. A compound of formula I, and/or a pharmaceutically acceptable salt thereof,



wherein when A is phenyl, B is acetoxy; when A is tert-butoxy, B is hydroxy; $n=2, 3, 4, 5, 6, 7, 8, 9, 10, 11$ or 12 ; wherein X is chosen from C_{1-6} alkyl, C_{1-6} alkylene and C_{1-6} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is independently chosen from oxygen, sulfur and nitrogen; or X is a single bond;

wherein M is chosen from aryl and heteroaryl;

wherein R is chosen from hydrogen, substituted or unsubstituted straight or branched C_{1-6} alkyl, hydroxy, substituted or unsubstituted straight or branched C_{1-6} alkoxy, thiol, substituted or unsubstituted straight or branched C_{1-6} alkylthio, C_{1-6} alkoxy- C_{1-6} alkyl, amino; substituted or unsubstituted straight or branched C_{1-6} mono- and di-alkylamino; aldehyde group, substituted or unsubstituted straight or branched C_{1-6} alkylcarbonyl, carboxyl, substituted or unsubstituted straight or branched C_{1-6} alkylcarboxyl, carbamoyl, substituted or unsubstituted straight or branched C_{1-6} alkylamide, C_{2-6} alkene, halogen, nitro and cyano;

wherein the substituent(s) on C_{1-6} straight chain or branched chain described herein is independently chosen from hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro and cyano.

2. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein $n=2, 3, 4, 5, 6, 7, 8, 9$ or 10 .

104

3. The compound and/or pharmaceutically acceptable salt thereof according to claim 2, wherein $n=2, 3, 4, 5, 6, 7$ or 8 .

4. The compound and/or pharmaceutically acceptable salt thereof according to claim 3, wherein $n=2, 3, 4$ or 5 .

5. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein X is chosen from C_{1-4} alkyl, C_{1-4} alkylene and C_{1-4} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is independently chosen from oxygen and sulfur; or X is a single bond.

6. The compound and/or pharmaceutically acceptable salt thereof according to claim 5, wherein X is chosen from C_{1-3} alkyl, C_{1-3} alkylene and C_{1-3} alkyl comprising at least one heteroatom, wherein the heteroatom is oxygen; or X is a single bond.

7. The compound and/or pharmaceutically acceptable salt thereof according to claim 6, wherein X is chosen from $-C=C-$, $-CH_2-CH_2-$, $-O-CH_2-$ and a single bond.

8. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the aryl is chosen from five membered to fourteen membered aryl.

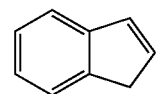
9. The compound and/or pharmaceutically acceptable salt thereof according to claim 8, wherein the aryl is chosen from five-membered aryl, six-membered aryl, nine-membered fused ring aryl, ten-membered fused ring aryl, thirteen-membered fused ring aryl and fourteen-membered fused ring aryl.

10. The compound and/or pharmaceutically acceptable salt thereof according to claim 9,

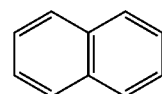
wherein the six-membered aryl is



wherein the nine-membered fused ring aryl is chosen from



wherein the ten-membered fused ring aryl is



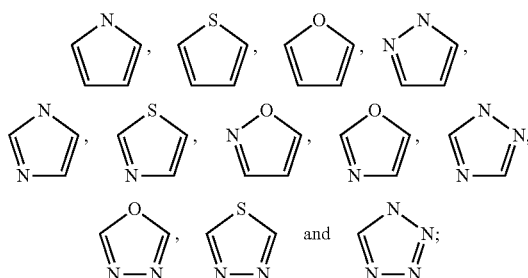
11. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the heteroaryl is chosen from heterocyclic aromatic ring comprising one, two, three or four heteroatoms in the ring, wherein the heteroatom(s) is independently chosen from nitrogen, oxygen and sulfur.

12. The compound and/or pharmaceutically acceptable salt thereof according to claim 11, wherein the heteroaryl is chosen from five membered to fourteen membered heterocyclic aromatic ring comprising one, two, three or four heteroatoms in the ring, wherein the heteroatom(s) is independently chosen from nitrogen, oxygen and sulfur.

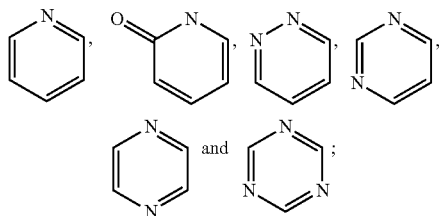
105

13. The compound and/or pharmaceutically acceptable salt thereof according to claim 12, wherein the heteroaryl is chosen from five-membered heterocyclic aromatic ring, six-membered heterocyclic aromatic ring, eight-membered fused heterocyclic aromatic ring, nine-membered fused heterocyclic aromatic ring and ten-membered fused heterocyclic aromatic ring, wherein the aromatic ring comprising one, two, three or four heteroatoms in the ring, wherein the heteroatom(s) is independently chosen from nitrogen, oxygen and sulfur.

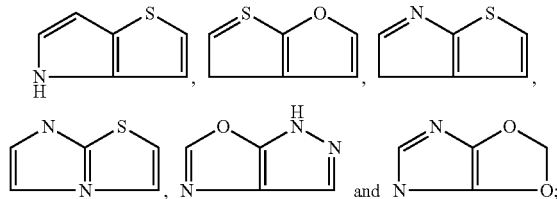
14. The compound and/or pharmaceutically acceptable salt thereof according to claim 13, wherein the five-membered heterocyclic aromatic ring is chosen from



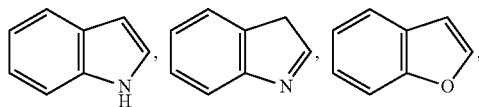
wherein the six-membered heterocyclic aromatic ring is chosen from



wherein the eight-membered fused heterocyclic aromatic ring is chosen from

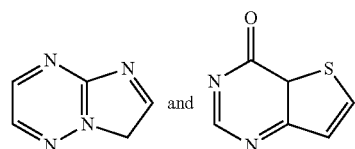
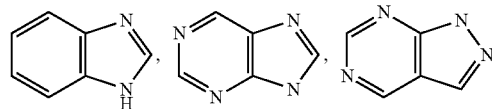


wherein the nine-membered fused heterocyclic aromatic ring is chosen from

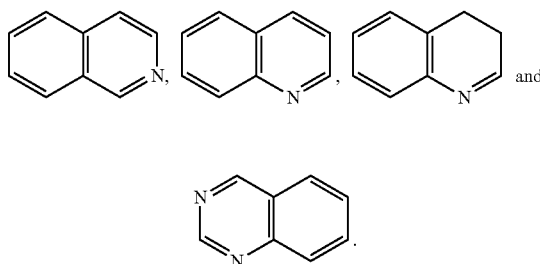


106

-continued



wherein the ten-membered fused heterocyclic aromatic ring is chosen from



15. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein R is chosen from hydrogen, substituted or unsubstituted straight or branched C₁₋₄ alkyl, hydroxy, substituted or unsubstituted straight or branched C₁₋₄ alkoxy, C₁₋₄ alkoxy-C₁₋₄ alkyl, thiol, substituted or unsubstituted straight or branched C₁₋₄ alkylthio, amino, substituted or unsubstituted straight or branched mono- and di-C₁₋₄ alkylamino, aldehyde group, substituted or unsubstituted straight or branched C₁₋₄ alkylcarbonyl, carboxyl, substituted or unsubstituted straight or branched C₁₋₄ alkylcarboxyl, carbamoyl, substituted or unsubstituted straight or branched C₁₋₄ alkylamide, C₂₋₄ alkene, halogen, nitro and cyano; wherein the substituent(s) on straight or branched C₁₋₄ alkyl is independently chosen from hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, fluorine, chlorine, bromine, nitro and cyano.

16. The compound and/or pharmaceutically acceptable salt thereof according to claim 15, wherein R is chosen from hydrogen, straight or branched C₁₋₄ alkyl, hydroxy, straight or branched C₁₋₄ alkoxy, thiol, straight or branched C₁₋₄ alkylthio, amino, straight or branched C₁₋₄ alkylamino, halogen, nitro and cyano.

17. The compound and/or pharmaceutically acceptable salt thereof according to claim 16, wherein R is chosen from hydrogen, hydroxyl, thiol, amino, fluorine, chlorine, bromine, nitro, cyano, methyl, ethyl, n-propyl, iso-propyl, methoxy, ethoxy, n-propoxy and iso-propoxy.

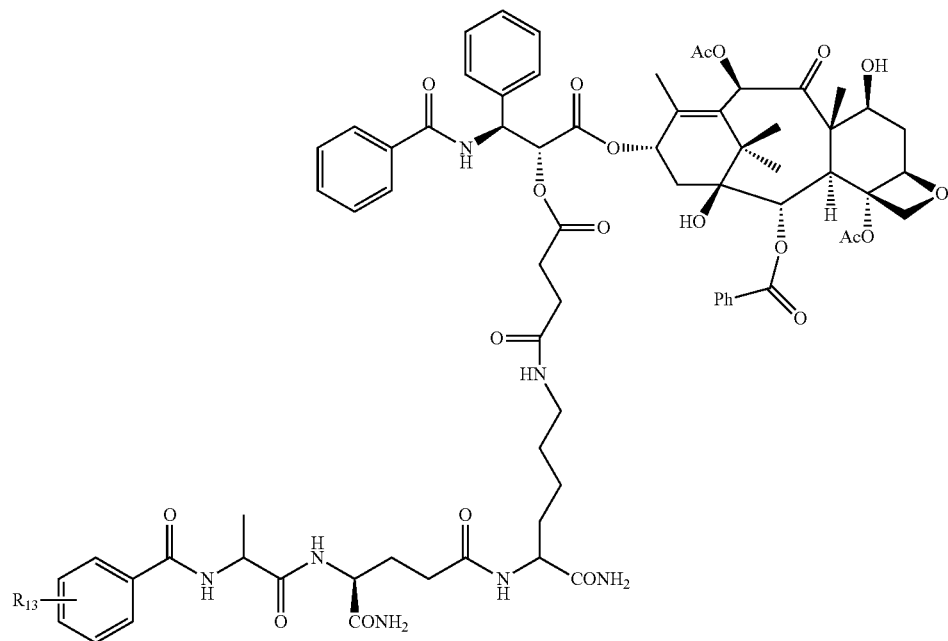
18. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from compounds of formula IA

The chemical structure of compound 10 is a complex molecule. It features a steroid core with several functional groups: an acetoxy (AcO) group at C-3, a hydroxyl (OH) group at C-14, and a phenylacetate (Ph-COO-) group at C-17. A long chain is attached to the steroid core via an ester linkage at C-17. This chain contains a secondary amide (HN) and a terminal amide (CONH₂). The chain is further substituted with a phenyl group (Ph) and a long alkyl chain. The structure is labeled with R₁₁ and includes stereochemical indicators (wedges and dashes) to show the 3D arrangement of atoms.

20. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from compounds of formula IC

The chemical structure of compound 10 is a complex molecule. It features a thienothiopyran core (a six-membered ring with a sulfur atom and an oxygen atom, fused to a five-membered ring with a sulfur atom). This core is substituted with a benzamide group (NH-C(=O)-C₆H₅) and a phenyl ester group (O-C(=O)-C₆H₅). A long aliphatic chain (CH₂)₆ is attached to the core via an amide linkage (NH-C(=O)-). This chain is further substituted with an amide group (NH-C(=O)-) and a carboxamide group (CONH₂). The structure also includes a thienothiopyran ring system with a sulfur atom and an oxygen atom, and a thienothiopyran ring system with a sulfur atom and an oxygen atom. The structure is highly complex and contains many functional groups, including amides, esters, and carboxamides.

IC



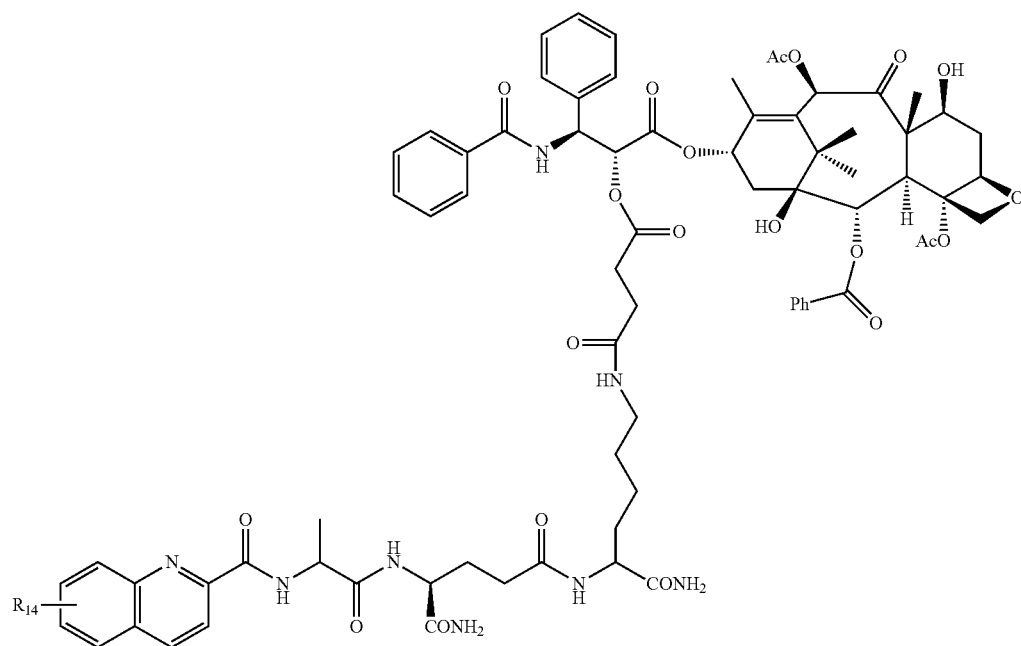
wherein R_{13} is at least one group independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkyl amino and C_{1-4} alkoxy- C_{1-4} alkyl.

wherein R_{14} is at least one group independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkyl amino and C_{1-4} alkoxy- C_{1-4} alkyl.

21. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from compounds of formula ID

22. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from compounds of formula IE

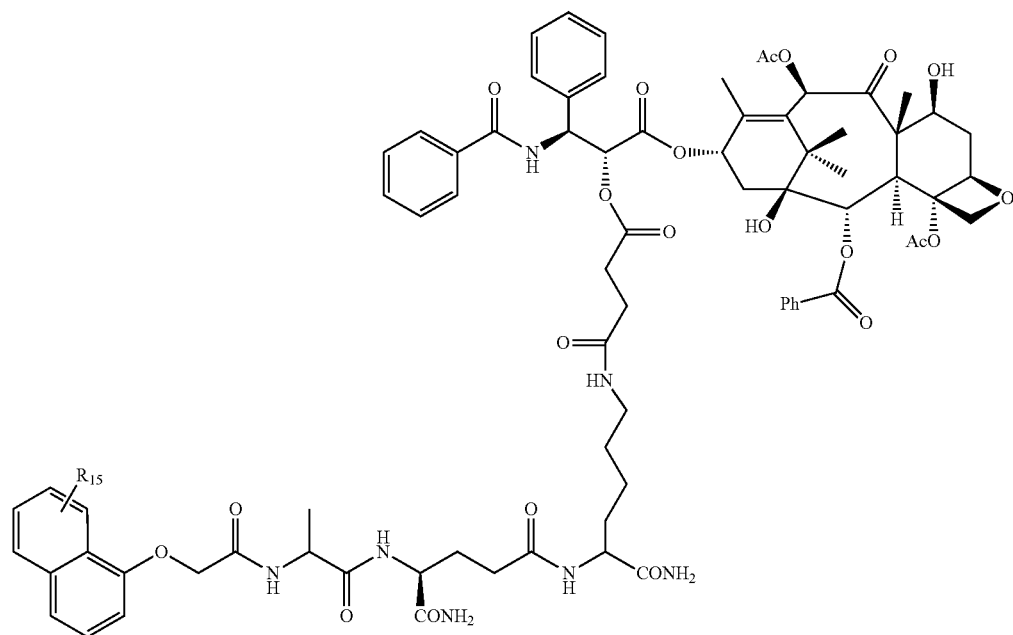
ID



111

112

IE



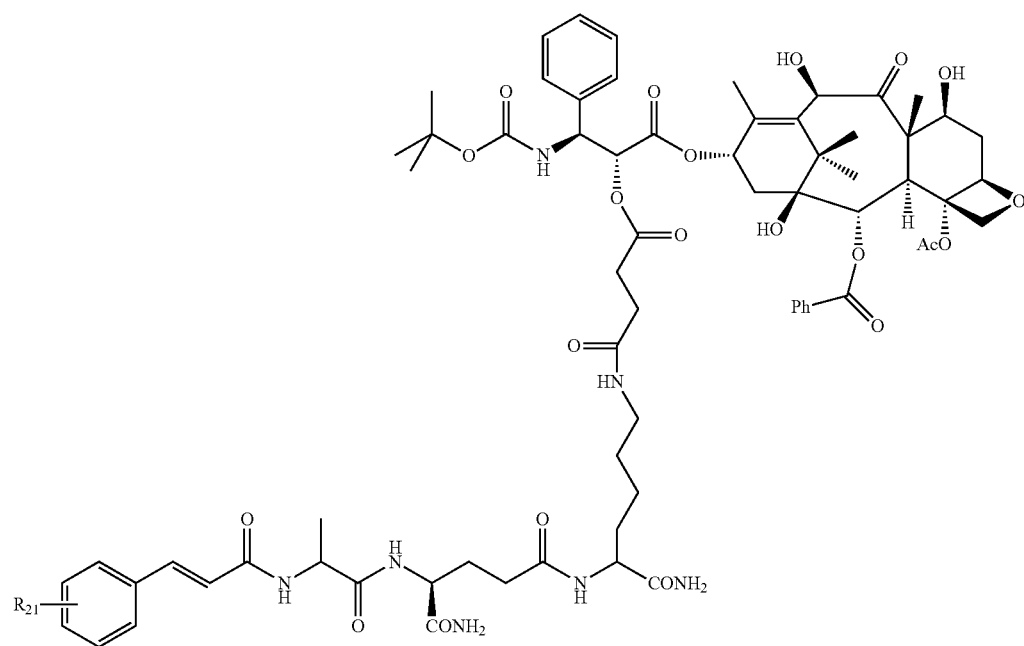
wherein R_{15} is at least one group independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkyl amino and C_{1-4} alkoxy- C_{1-4} alkyl.

wherein R_{21} is at least one group independently chosen from hydrogen, hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkyl amino and C_{1-4} alkoxy- C_{1-4} alkyl.

23. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from compounds of formula IF

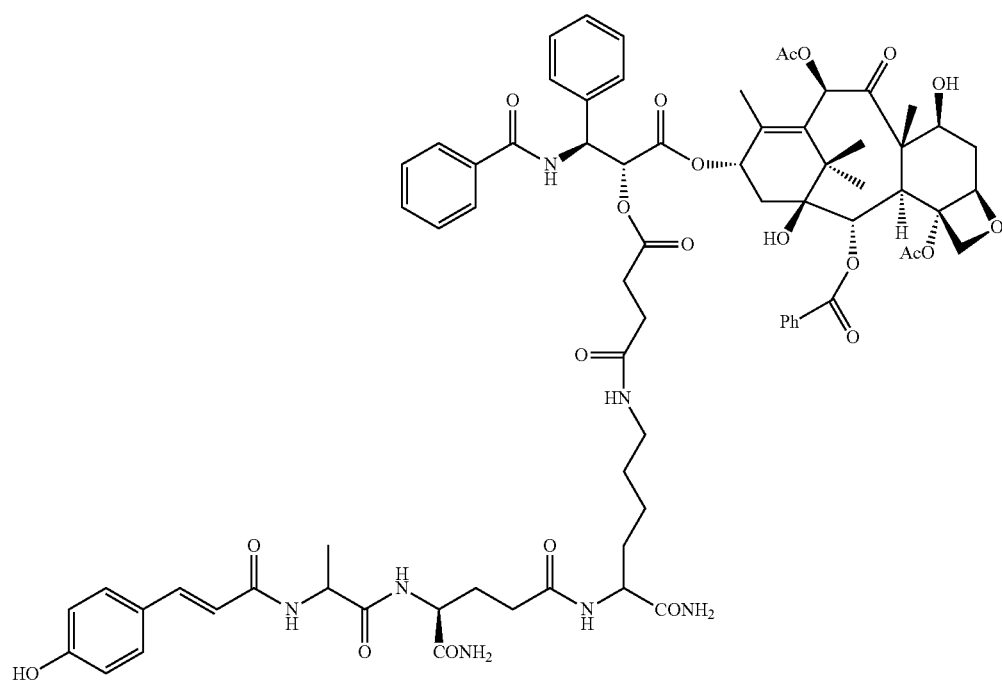
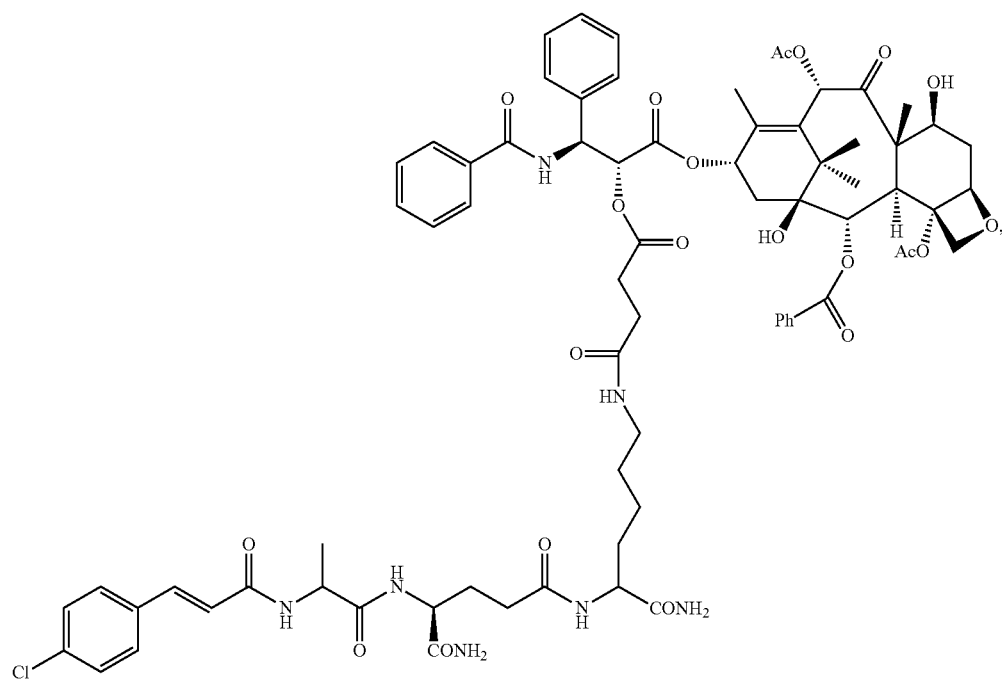
24. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is chosen from:

IF



113

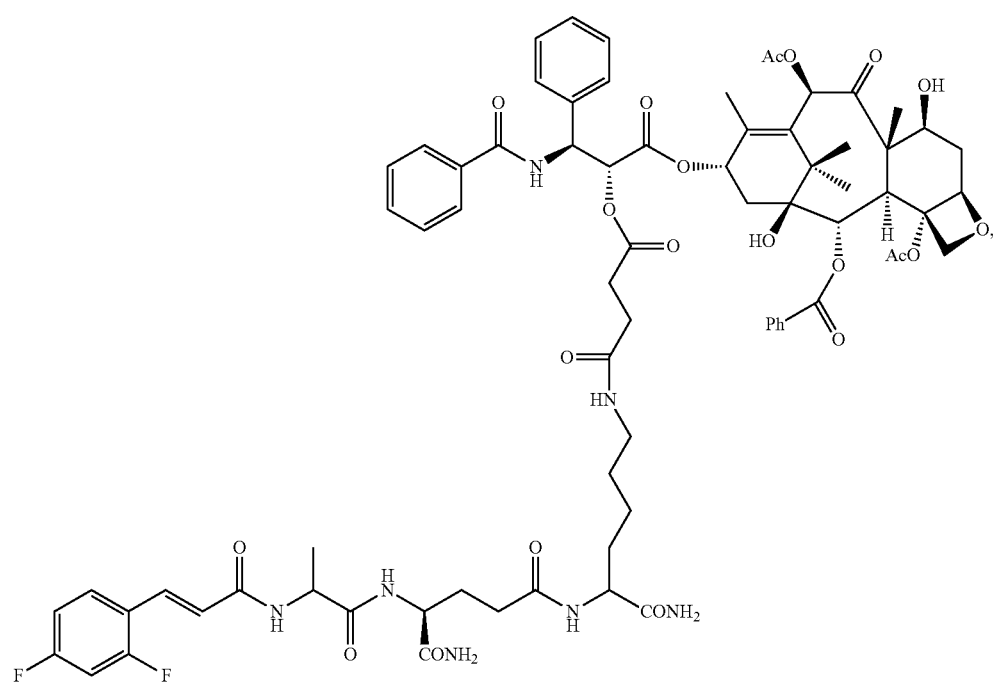
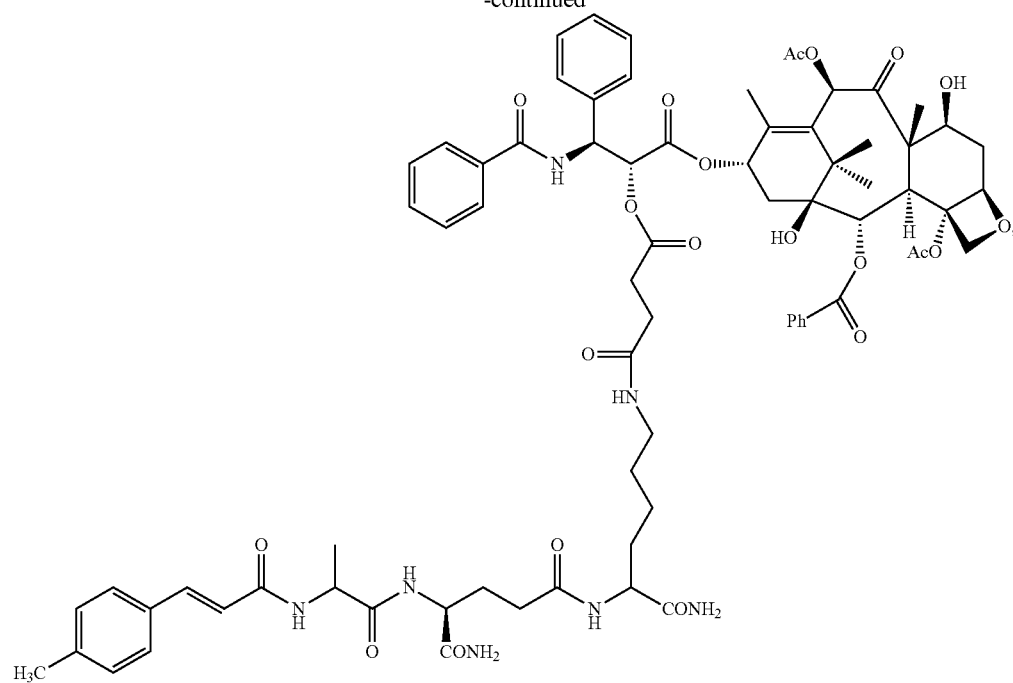
114



115

116

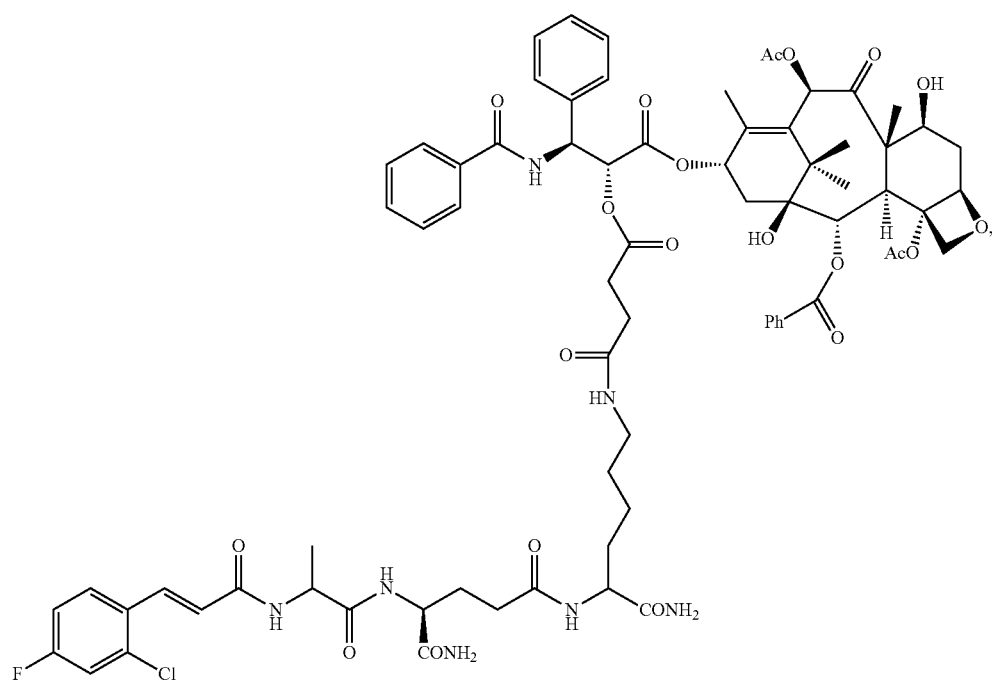
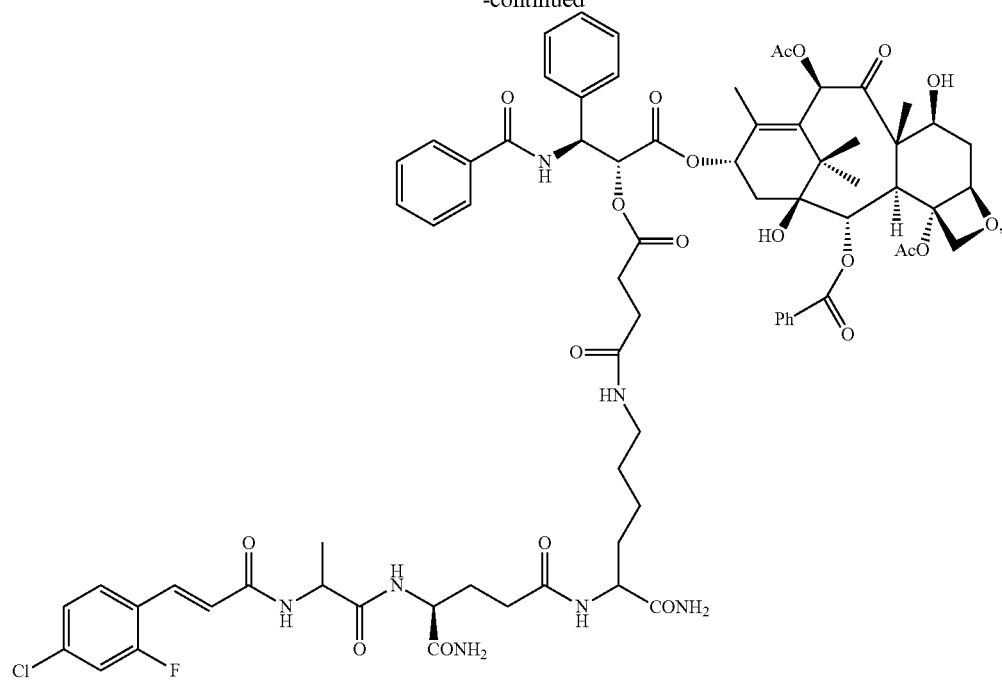
-continued



117

118

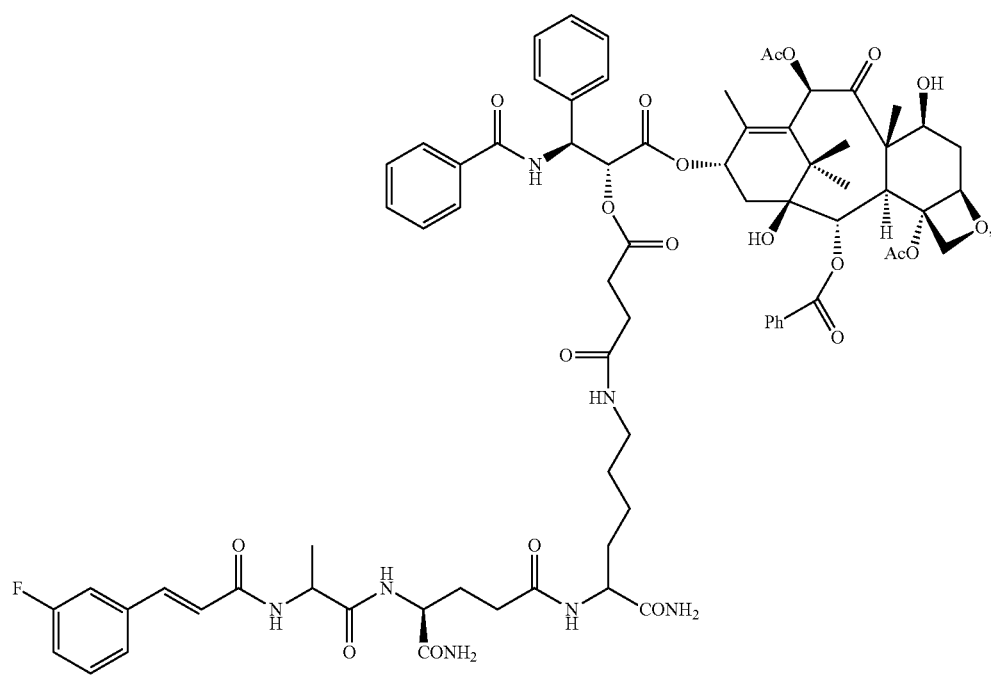
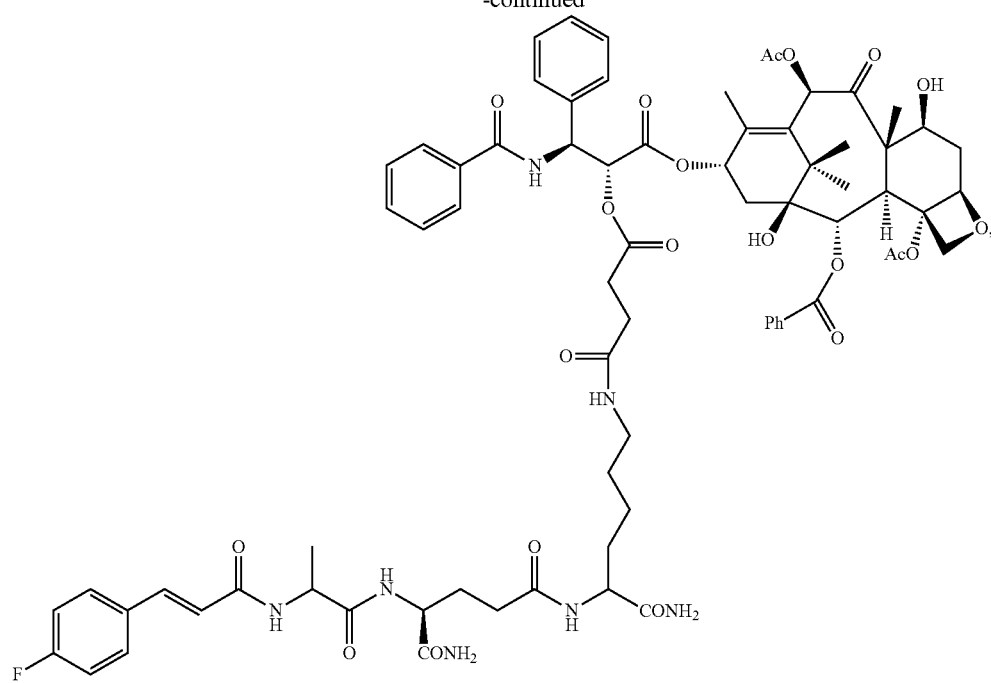
-continued



119

120

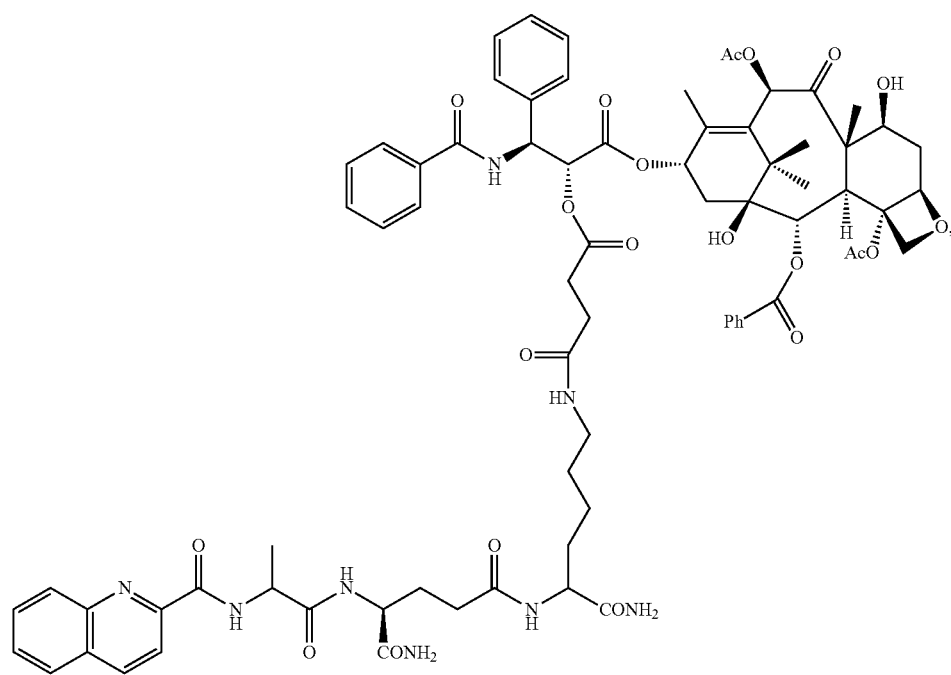
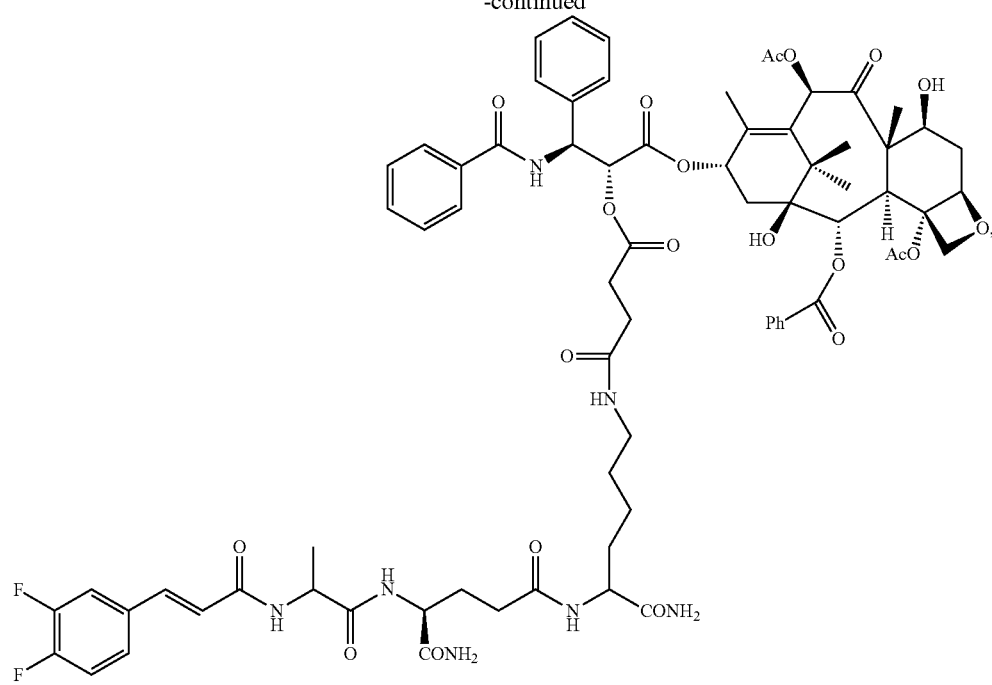
-continued



121

122

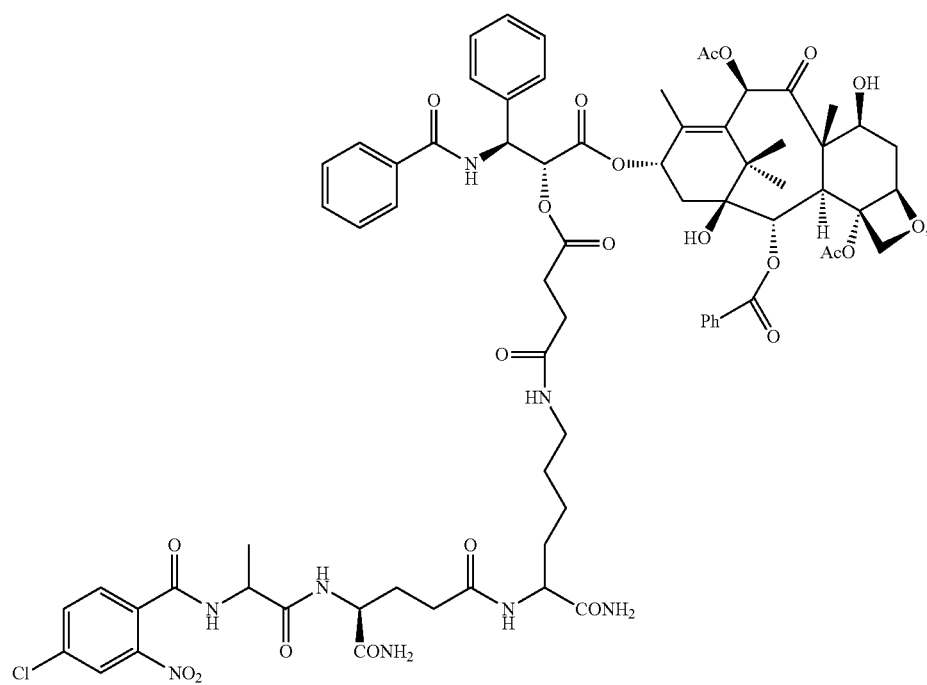
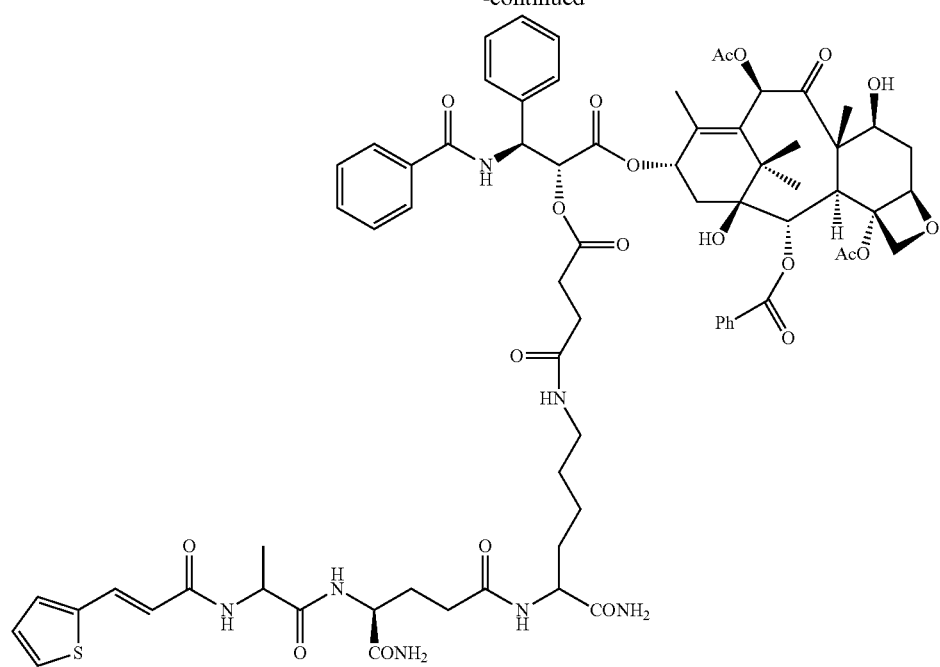
-continued



123

124

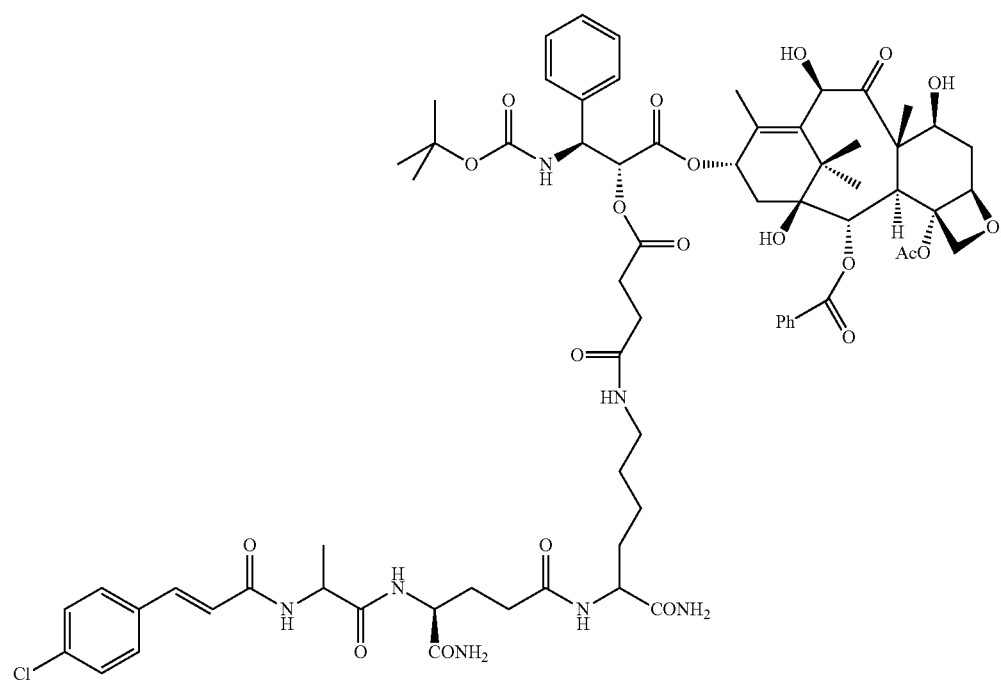
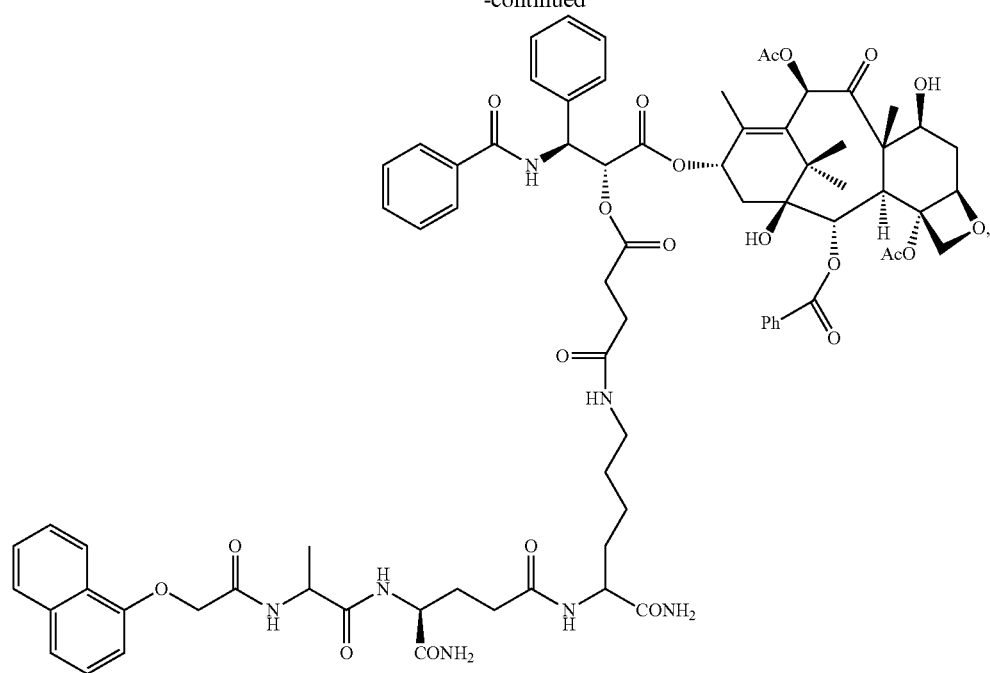
-continued



125

126

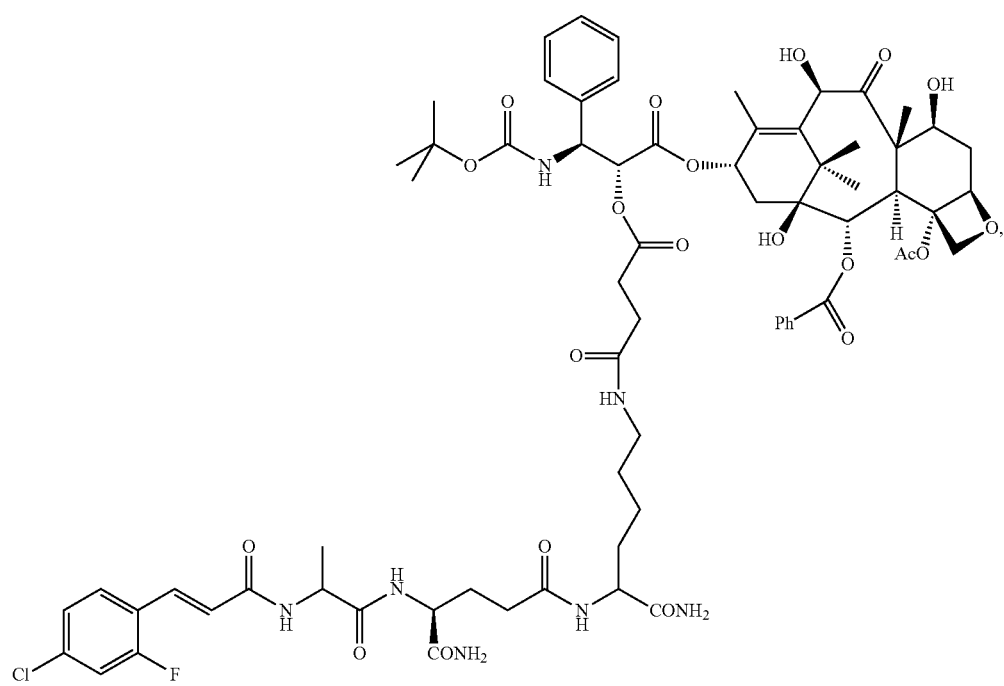
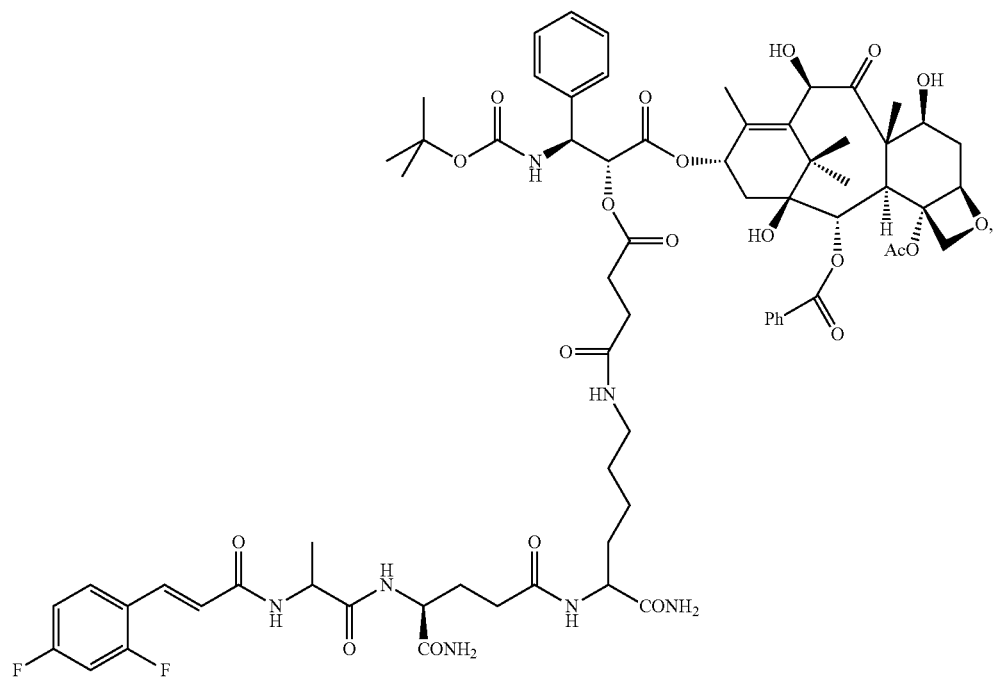
-continued



127

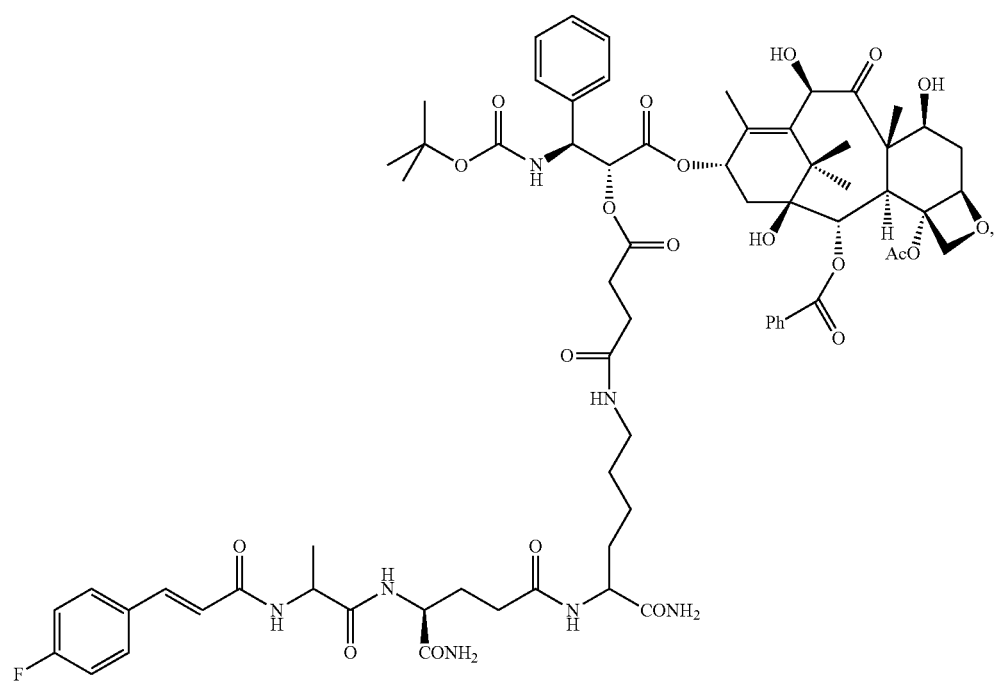
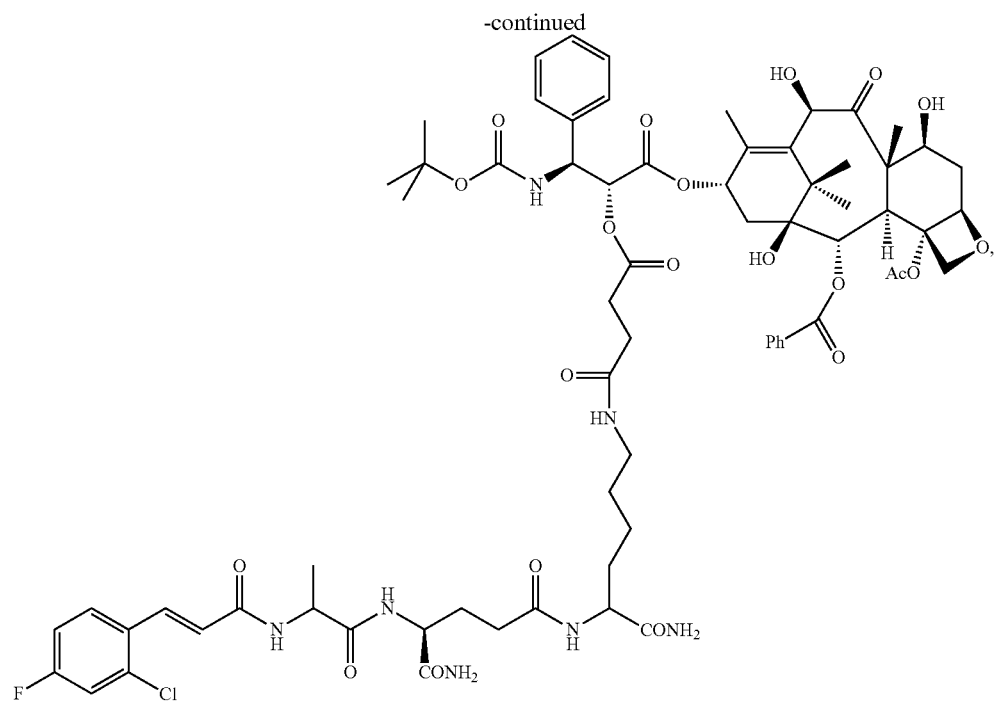
128

-continued



129

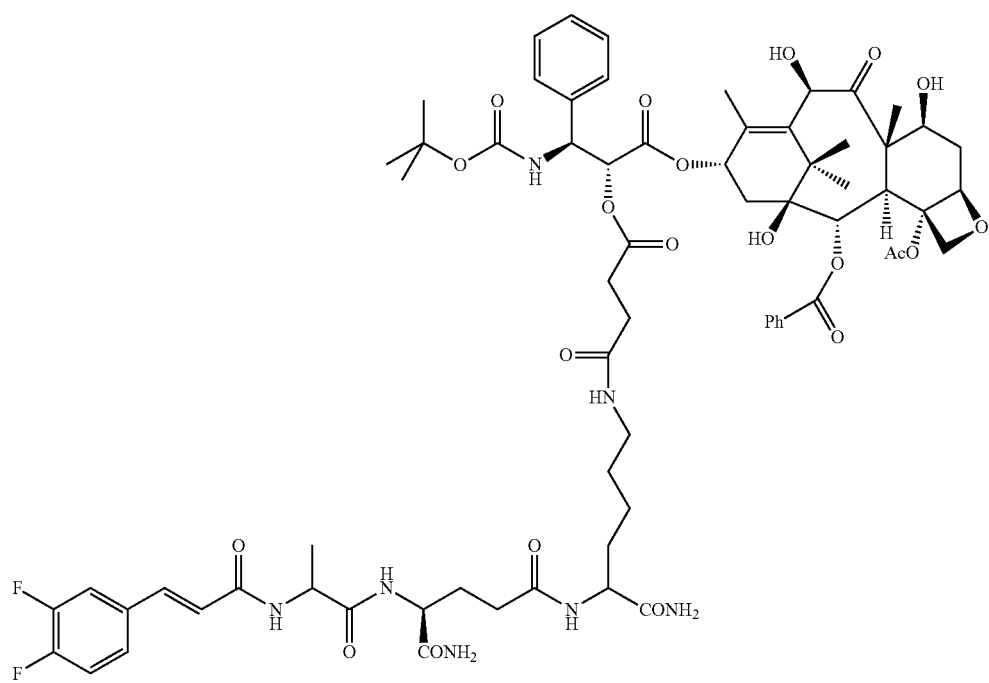
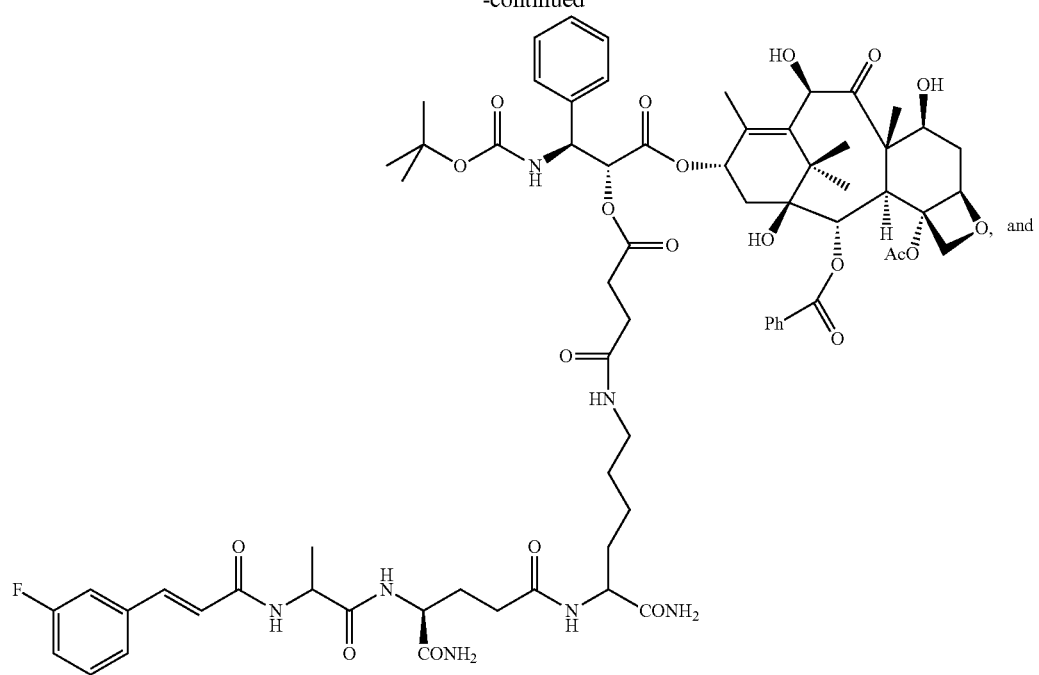
130



131

132

-continued



133

25. The compound and/or pharmaceutically acceptable salt thereof according to claim 1, wherein the pharmaceutically acceptable salt is chosen from hydrochloride, hydrobromide, sulfate, phosphate, nitrate, oxalate, fumarate, maleate, succinate, citrate, tartrate, mesylate and p-toluenesulfonate.

26. A pharmaceutical composition comprising the compound and/or pharmaceutically acceptable salt thereof according to claim 1 and at least one pharmaceutically accepted carrier.

27. A method for treating immune regulation comprising administering to the subject a therapeutic amount of the compound and/or pharmaceutically acceptable salt thereof according to claim 1.

28. A method for preventing and/or treating cancer comprising administering to the subject a therapeutic amount of compound and/or pharmaceutically acceptable salt thereof according to claim 1

wherein the cancer is chosen from melanoma, gastric cancer, lung cancer, breast cancer, renal cancer, liver cancer, oral cavity epidermal carcinoma, cervical cancer, oophoroma, pancreatic cancer, prostatic cancer and colonic cancer.

29. A method for preparing the compound and/or pharmaceutically acceptable salt thereof according to claim 1, comprising:

- 1) synthesizing Paclitaxel-2'-O-alkane-di-acid monoester or docetaxel-2'-O-alkane-di-acid monoester in liquid-phase;
- 2) synthesizing Muramyl dipeptide Analogue on solid-phase or in liquid-phase;
- 3) synthesizing conjugates of Muramyl Dipeptide Analogue and paclitaxel, or conjugates of Muramyl Dipeptide Analogue and docetaxel in liquid-phase.

30. The method according to claim 29, wherein the step 1) of the method for preparing paclitaxel-2'-O-alkane-di-acid monoester comprises:

- (1) dissolving Paclitaxel, alkane-di-anhydride and 4-N,N-dimethyl pyridine in pyridine, and stirring for 4 h at room temperature;
- (2) diluting the pyridine solution with acetic ether, then washing the acetic ether layer with saturated CuSO_4 aqueous solution and H_2O sequentially;
- (3) separating and concentrating the acetic ether layer under vacuum, adding abundant water into the residue, then filtering and lyophilizing the white solid to obtain paclitaxel-2'-O-alkane-di-acid monoester.

31. The method according to claim 29, wherein step 1) of the method for preparing docetaxel-2'-O-alkane-di-acid monoester comprises:

- (1) dissolving docetaxel, alkane-di-anhydride and 4-N,N-dimethyl pyridine in N,N-dimethylformamide, and stirring for 2 h at room temperature;
- (2) diluting the N,N-dimethylformamide solution with dichloromethane, then washing the dichloromethane layer with 2N HCl aqueous solution and H_2O sequentially;
- (3) separating and concentrating the dichloromethane layer under vacuum, dissolving the residue in a small amount of methanol, then adding abundant water into the residue, then filtering and lyophilizing the white solid to obtain docetaxel-2'-O-alkane-di-acid monoester.

32. The method according to claim 29, wherein step 2) of the method for preparing muramyl dipeptide analogue comprises:

134

1) Solid-phase synthesis:

- (1) Firstly, synthesizing the intermediate Fmoc-D-iso-Gln-OH in liquid-phase;
- (2) Then, introducing Fmoc-L-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-L-Ala-COOH and carboxylic acid to the solid phase carrier of aminoresin of Rink-Amide AM by solid-phase synthesis wherein the condensation reaction is a conventional amide condensation reaction, the condensation reaction is reacted completely by adding the excess amount of the above three amino acids or anyone carboxylic acid and any condensing agent of HATU or HBTU, BOP or PyBOP, and obtaining muramyl dipeptide analogue by the steps comprising washing and cleaving the resin thoroughly, and purifying the crude product;

2) Liquid-phase synthesis:

- (1) Firstly, synthesizing the intermediate of Boc-D-Glu(OBzl)- NH_2 and Boc-Lys(Z)- NH_2 ;
- (2) Then, synthesizing the dipeptide fragment of Boc-Ala-D-Glu(OBzl)- NH_2 and the tripeptide fragment of R-Ala-D-Glu(OBzl)- NH_2 by the active ester method, and removing the Bzl protective group by the acetic acid solution of hydrobromic acid or other acid or base, synthesizing the tetrapeptide of R-Ala-D-iso-Gln-Lys(Z)- NH_2 by the active ester method;
- (3) At last, removing the Z protective group by the mixed solution of boron trifluoride ethylether, trifluoroacetic acid and ethanethiol (v/v/v=9:9:2) to obtain the crude product, and purifying the crude product to obtain muramyl dipeptide analogue.

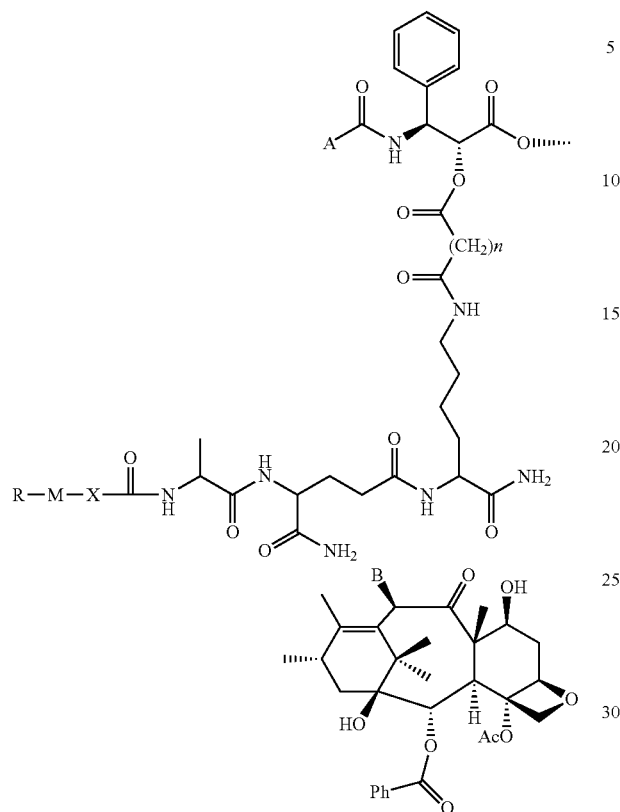
33. The method according to claim 32, wherein the amino acids of Fmoc-L-Lys(Boc)-COOH, Fmoc-D-iso-Gln-COOH, Fmoc-L-Ala-COOH in the solid-phase synthesis can be replaced by any natural or unnatural amino acid.

34. The method according to claim 29, wherein the method for preparing the conjugates of muramyl dipeptide analogue and paclitaxel or conjugates of muramyl dipeptide analogue and docetaxel comprises:

- 1) Firstly, dissolving paclitaxel-2'-O-alkane-di-acid monoester or docetaxel-2'-O-alkane-di-acid monoester, HOSu and DIC with molar ratio (2:1-1:2) in the solution of dimethyl sulfoxide or N,N-dimethyl formamide or N-methyl pyrrolidone, then allowing the solution to react for 1-10 hours at the temperature of -20°C . to $+50^\circ\text{C}$.;
- 2) Then, adding equimolar proportions of muramyl dipeptide analogue to the solution of dimethyl sulfoxide or N,N-dimethyl formamide or N-methyl pyrrolidone, adjusting the pH of the reaction system to 6 to 8 by alkalescence reagent N-methyl morpholine, allowing the reaction to continue for 1-10 hours, obtaining the conjugate after reaction completed;
- 3) At last, adding any one of water, methanol, ethanol, diethyl ether, petroleum ether and ethyl butyl ether to the reaction solution, and filtering the precipitated solid, and purifying the crude product by preparative HPLC or recrystallization and obtaining the target product.

135

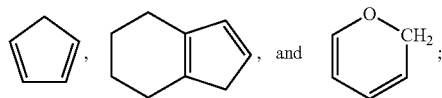
35. A compound of formula I, and/or a pharmaceutically acceptable salt thereof,



wherein when A is phenyl, B is acetoxy; when A is tert-butoxy, B is hydroxy; $n=2, 3, 4, 5, 6, 7, 8, 9, 10, 11$ or 12 ;

136

wherein X is chosen from C_{1-6} alkyl, C_{1-6} alkylene and C_{1-6} alkyl comprising at least one heteroatom, wherein the at least one heteroatom is independently chosen from oxygen, sulfur and nitrogen; or X is a single bond; wherein M is chosen from



wherein R is chosen from hydrogen, substituted or unsubstituted straight or branched C_{1-6} alkyl, hydroxy, substituted or unsubstituted straight or branched C_{1-6} alkoxy, thiol, substituted or unsubstituted straight or branched C_{1-6} alkylthio, C_{1-6} alkoxy- C_{1-6} alkyl, amino; substituted or unsubstituted straight or branched C_{1-6} mono- and di-alkylamino; aldehyde group, substituted or unsubstituted straight or branched C_{1-6} alkylcarbonyl, carboxyl, substituted or unsubstituted straight or branched C_{1-6} alkylcarboxyl, carbamoyl, substituted or unsubstituted straight or branched C_{1-6} alkylamide, C_{2-6} alkene, halogen, nitro and cyano; wherein the substituent(s) on C_1 - C_6 straight chain or branched chain described herein is independently chosen from hydroxyl, thiol, amino, aldehyde group, carboxyl, carbamoyl, halogen, nitro and cyano.

* * * * *